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UNIVERSITY OF ALBERTA

EFFECTS OF INTESTINAL TRANSPLANTATION AND CYCLOSPORINE ON SMALL INTESTINAL FUNCTION IN THE RAT

BY

David L. Sigalet ©

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF **Doctor of Philosophy**

IN

Experimental Surgery

DEPARTMENT OF SURGERY

EDMONTON, ALBERTA

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THE UNDERSIGNED CERTIFY THEY HAVE READ, AND RECOMMEND TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH FOR ACCEPTANCE, A THESIS ENTITLED **EFFECTS OF INTESTINAL TRANSPLANTATION AND CYCLOSPORINE ON SMALL INTESTINAL FUNCTION IN THE RAT** SUBMITTED BY **DAVID L. SIGALET** IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF **DOCTOR OF PHILOSOPHY** IN **EXPERIMENTAL SURGERY**.

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Alan B.R. Thomson (Committee Member)

DEDICATION

THIS THESIS IS DEDICATED TO MY FATHER,

JOHN DONALD (JACK) SIGALET (1933-1990),

FROM HIS LOVING SON, THE DOCTOR DOCTOR

ABSTRACT

These studies examined the effects of the immune suppressant cyclosporine A (CsA) and small intestinal transplantation (SIT) on intestinal function in inbred strains of rats. Our hypothesis was that both SIT and CsA would reduce the absorption of nutrients by the intestine. Orthotopic SIT was performed by transplanting the entire small intestine. Transplanted animals were followed for 60 days. CsA was given subcutaneously and orally in varying dosages to normal and transplanted animals. CsA in high doses reduced weight gain and, when given orally, reduced energy and fat absorption from the diet. CsA induced a consistent pattern of reduced in vitro active glucose uptake and a route-dependent reduction in in vitro fatty acid uptake. Syngeneic SIT (Brown Norway rats (BN→BN)) and allogeneic SIT (BN→Lewis) each reduced weight gain, and dietary nutrient absorption. SIT resulted in a consistent pattern of increased active glucose transport, but reduced glucose-stimulated intestinal short circuit current (Isc) and reduced conductance. This was accompanied by an increase in the in vivo permeability of the intestine to mannitol, but not to lactulose. The passive uptake of fatty acids varied with the strain of rats used, but tended to increase following SIT. The effects of syngeneic SIT on intestinal permeability to polyethylene glycol-400 and ^{51}CrEDTA also varied with the strain of rat, while allogeneic SIT resulted in an increased permeability to all of the probes used.

These findings demonstrate that CsA reduces the activity of the sodium-glucose cotransporter of the intestinal enterocyte, probably by a direct pharmacological inhibition, and by reducing the number of carrier proteins. It is further suggested that CsA alters the lipid composition of the brush border membrane of the enterocyte. The uptake data show that SIT induces an increase in intestinal permeability and a decrease in conductance, possibly mediated by denervation of the intestine, and these reduce glucose-stimulated Isc. The increase in sodium-glucose cotransporter activity demonstrated by the increased rate of

active glucose transport following SIT is likely compensatory, representing an adaptive response of the denervated intestine. The morphological data confirm that transplanted intestine can also adapt morphologically.

These adaptive changes in nutrient uptake and morphology serve to normalize the in vivo parameters of weight gain and nutrient absorption following CsA treated and SIT. It will be necessary to confirm the adaptive response following SIT in large animal models such as the pig, prior to performing SIT in man. These findings are useful in planning therapy for patients with preexisting intestinal absorption defects who may require CsA, and suggest that further research is required to determine the capacity of transplanted intestine to maintain adequate nutrition.

ACKNOWLEDGEMENTS

To the uninitiated, research may seem to be a dull exercise in accumulating facts. To me, research has become a process of falling in love with ideas, and then attempting to prove them, while attempting to remain impartial. I am profoundly grateful that I have had the opportunity to experience this.

The cooperation provided to me by the Surgical-Medical Research Institute of the Department of Surgery and the Division of Gastroenterology of the Department of Medicine, both at the University of Alberta, are an enviable example of what a university should be. The creative milieu of these sections of the Faculty of Medicine provided both the stimulus and the facilities for these studies.

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Finally, no job is finished until the paper work is complete. Special thanks to Dawne Colwell for the graphs and slides. The expert and timely assistance of Rosemarie Henley, Susan Evans-Davies, and Colleen Gardner was very much appreciated.

TABLE OF CONTENTS

CHAPTER PAGE

I. INTRODUCTION: EXPERIMENTAL DESIGN AND RATIONALE 1

Bibliography 5

II. SMALL BOWEL TRANSPLANTATION: PAST, PRESENT AND FUTURE 7

Bibliography 27

III. CYCLOSPORINE EFFECTS ON NORMAL BOWEL 36

Bibliography 44

IV. CYCLOSPORINE REDUCES NUTRIENT ABSORPTION IN NORMAL RATS 45

Bibliography 60

V. FUNCTIONAL PROPERTIES OF SMALL INTESTINE AFTER SYNGENEIC TRANSPLANTATION IN THE RAT 62

Bibliography 83

VI. INTESTINAL FUNCTION FOLLOWING ALLOGENEIC SMALL INTESTINAL TRANSPLANTATION IN THE RAT 87

Bibliography 112

VII. THE EFFECTS OF CYCLOSPORINE AND INTESTINAL TRANSPLANTATION ON INTESTINAL FUNCTION IN THE RAT 115

Bibliography 131

LIST OF TABLES

TABLE PAGE

III-1 IN VIVO AND IN VITRO EFFECTS OF CYCLOSPORINE ON INTESTINAL FUNCTION IN THE RAT. 42

III-2 EFFECT OF CYCLOSPORINE ON LIPID UPTAKE. 43

IV-1 CYCLOSPORINE DOSES. 54

IV-2 ANIMAL CHARACTERISTICS FOLLOWING CYCLOSPORINE TREATMENTS. 55

IV-3 CYCLOSPORINE EFFECTS ON ACTIVE GLUCOSE UPTAKE IN NORMAL RATS. 56

IV-4 CYCLOSPORINE EFFECTS ON LIPID UPTAKE IN NORMAL RATS. 57

IV-5 CYCLOSPORINE EFFECTS ON INTESTINAL MORPHOLOGY IN NORMAL RATS. 58-59

V-1 ANIMAL CHARACTERISTICS FOLLOWING SYNGENEIC SMALL INTESTINAL TRANSPLANTATION. 75

V-2 INTESTINAL PERMEABILITY FOLLOWING SYNGENEIC SMALL INTESTINAL TRANSPLANTATION. 76

V-3 INTESTINAL TRANSPLANTATION: EFFECTS IN THE KINETICS OF GLUCOSE UPTAKE. 77

V-4 IN VITRO LIPID UPTAKE FOLLOWING SYNGENEIC SMALL INTESTINAL TRANSPLANTATION. 78

V-5 INTESTINAL SHORT CIRCUIT CURRENT FOLLOWING SMALL INTESTINAL TRANSPLANTATION. 79

V-6 INTESTINAL MORPHOLOGY FOLLOWING SYNGENEIC SMALL INTESTINAL TRANSPLANTATION. 80-81

VI-1 ANIMAL CHARACTERISTICS AND NUTRIENT ABSORPTION FOLLOWING ALLOGENEIC SMALL INTESTINAL TRANSPLANTATION. 103

VI-2 PASSIVE PERMEABILITY OF THE SMALL INTESTINE FOLLOWING ALLOGENEIC INTESTINAL TRANSPLANTATION. 104

VI-3 IN VITRO NUTRIENT UPTAKE FOLLOWING ALLOGENEIC INTESTINAL TRANSPLANTATION. 105

VI-4 IN VITRO FATTY ACID UPTAKE FOLLOWING ALLOGENEIC INTESTINAL TRANSPLANTATION. 106

VI-5 ELECTROPHYSIOLOGICAL PARAMETERS FOLLOWING ALLOGENEIC INTESTINAL TRANSPLANTATION. 107

VI-6 INTESTINAL MORPHOLOGY FOLLOWING ALLOGENEIC INTESTINAL TRANSPLANTATION. 108-109

LIST OF FIGURES

FIGURE PAGE

V-1 INTESTINAL SHORT CIRCUIT CURRENT: CHANGES WITH GLUCOSE AND THEOPHYLLINE FOLLOWING SYNGENEIC SMALL INTESTINAL TRANSPLANTATION. 82

V1-1 WEIGHT GAIN AFTER ALLOGENEIC SMALL INTESTINAL TRANSPLANTATION. 110

VI-2 SHORT CIRCUIT CURRENT: CHANGES WITH GLUCOSE AND THEOPHYLLINE FOLLOWING ALLOGENEIC SMALL INTESTINAL TRANSPLANTATION. 111

LIST OF ABBREVIATIONS

Apparent Michaelis Constant	(Km*)
Brown Norway	(BN)
Cyclosporine A	(CsA)
Graft-Versus-Host-Disease	(GVHD)
Host-Versus-Graft	(HVG)
Jejunal Maximal Transport Rate	(Vmax)
Lewis	(Lew)
Short Bowel Syndrome	(SBS)
Short Circuit Current	(Isc)
Small Intestinal Transplantation	(SIT)
Total Parenteral Nutrition	(TPN)

CHAPTER I

INTRODUCTION: EXPERIMENTAL DESIGN AND RATIONALE

The ability to supply the nutritional requirements of patients using total parenteral nutrition (TPN) has been a major advance in treating conditions where gastrointestinal absorptive function is compromised (1). The most extreme example of this is in the short bowel syndrome (SBS) that results from massive losses of the small bowel. Long-term TPN, administered at home, has allowed some patients to return to work and many more to live a reasonably normal lifestyle. However, TPN has many disadvantages, including its cost, restriction of the patient's life style, and the continual risk of catheter sepsis (1-3). These disadvantages are accentuated in the neonatal and pediatric age groups. Neonates are particularly prone to hepatic complications following prolonged TPN (4), while older children have high nutrient requirements and problems with compliance (5,6).

Small intestinal transplantation (SIT) as a treatment for SBS has been considered since the early twentieth century. However, it is only recently that advances in immune suppression have resulted in success with large animal models of small intestinal allografts (7,8). Similar regimens have had limited success in attempts at SIT in humans (9-11). SIT, with combined liver transplantation, has proven successful in six cases to date (11,12). These studies have elicited a great deal of interest in the field of SIT. However, the majority of this work has focused either on the technical aspects of transplantation or the immune suppression regimen required. For SIT to be considered for clinical use, it will be necessary to quantify the functional capacity of the intestine, and to determine the mechanisms underlying any deficiencies noted.

This thesis is written in "paper" format, with each chapter being an independent paper. Chapter II is a review of the previous literature, while chapters III through VI deal with the experimental studies performed. Chapter VII summarizes these findings, discusses the mechanisms which may be involved, and offers suggestions for future studies.

The studies reviewed in chapter II suggest that while experimental animals do grow following SIT, dietary nutrient absorption is impaired (13-15). There are no studies examining the mechanism of this impairment. The studies which have been done examining nutrient transport at the cellular level were concerned with the acute effects of rejection and the effects of perioperative ischemia (16,17). In addition to the effects of transplantation and the associated immune interactions of allogeneic transplantation, intestinal function may also be affected by cyclosporine A (CsA), which remains the mainstay of immune suppressive regimens for experimental and clinical transplantation. CsA has been used in the majority of the successful cases reported to date (10-12). Indirect evidence suggests that CsA impairs ion and chloride absorption, in both normal and autotransplanted bowel (18), but this has not been examined directly. CsA is known to reduce bile secretion in rats (19), and to reduce the activity of the sodium-glucose cotransporter in cell culture (20).

Given these suggestions that transplantation and CsA treatment may affect nutrient absorption by the intestine, the following experiments were designed. The rat was chosen as the experimental animal for these studies. Syngeneic strains of rats have the advantages of defined genetic status, which ensures consistent metabolic and immune responses (21). The CsA requirements for immune suppression have been clearly determined (14,16), the model is not unduly expensive to work with, and the transport characteristics of the bowel have been well described (22).

Intestinal function was monitored by following the parameters of weight gain and feed intake for a 60 day period. Under these circumstances, weight gain is an accurate reflection of nutrient availability (23). Over days 50 to 53 of the study, a balance study was performed, quantifying feed intake and fecal output in metabolic cages. Using bomb calorimetry (24), the energy content of feed and feces was determined and the absorption calculated directly. Dietary fat absorption was calculated in a similar fashion. Intestinal permeability has been used as a marker for rejection following SIT (25), but the effects of transplantation and controlled rejection have not been systematically examined. Accordingly, intestinal permeability was measured using a series of probes of varying sizes (mannitol, lactulose, ^{51}Cr-EDTA and polyethylene glycol-400) at day 55.

These in vivo correlations of nutrient handling were then compared with intestinal function in vitro. At day 60 of the studies, nutrient uptake by the intestinal mucosa was measured directly, and the kinetics of D-glucose active transport and the passive uptake of fatty acids calculated (26). For the studies involving transplanted bowel intestinal short circuit current, and the response to glucose and theophylline was measured (21). These parameters are a reflection of the net transmural flux of glucon and ions, and also assess the secretory status of the bowel (21). Finally, standard morphology was performed.

These studies examined the general well-being of the animal, the absorption of nutrients from the diet, and the functional capacity of the enterocyte. Thus, the effects of SIT on each level of intestinal function could be determined.

The experiments conducted first examined the effects of CsA on nutrient handling by normal bowel (Chapter III). The dose used (15 mg/kg) is typical of the dose required for immune suppression following fully allogeneic SIT. Having determined that CsA did reduce glucose and fatty acid uptake, the effects of varying the dose, and route of administration was then determined in chapter IV. The effects of transplantation alone, without the possibility of rejection were examined using a two-stage model of total orthotopic small intestinal transplantation between Lewis (Lew) rats. This model was chosen because it causes less weight loss after transplantation, and is technically easier than a one-stage transplant (27). Normal Lew rats, Lew→Lew transplants, not treated with CsA, and Lew→Lew transplants treated with CsA (15 mg/kg) were compared (Chapter V). Finally, the effects of SIT between fully allogeneic strains was examined in the study outlined in chapter VI. Brown Norway (BN) rat bowel was transplanted into Lew recipients. BN bowel was shown to have different transport characteristics in vitro and so the function following allogeneic transplantation was compared with normal BN bowel, and BN→BN syngeneic transplants. However, BN and Lew rats grow at different rates, and so the weight gain following BN→Lew transplantation was compared to syngeneic Lew→Lew transplanted animals. Because of the effects of CsA, all animals received identical treatment with 15 mg/kg.

The implications of these findings, and suggestions for future research were then summarized in chapter VII.

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CHAPTER II

SMALL BOWEL TRANSPLANTATION: PAST, PRESENT AND FUTURE[1]

INTRODUCTION

The short bowel syndrome (SBS) that results from massive losses of the small bowel continues to be a difficult problem despite the common use of long-term total parenteral nutrition (TPN). TPN is expensive (annual cost of TPN was greater than $50,000 per year in 1980) (1), limits the lifestyle of the patient and their families (2), and requires long-term venous access. Long-term TPN in children is even more problematic, with increased nutrient requirements, difficulty with patient compliance, and the risk of associated liver damage, especially in the very young infant (3). Because of these factors, the life long mortality from direct complications of long-term TPN in the pediatric age group is 15% (4). Massive resections of the small bowel in children most commonly result from necrotizing enterocolitis, volvulus, and strangulated abdominal wall defects (4). In the adult population, massive bowel resection most commonly result from mesenteric vascular accidents, inflammatory bowel disease, and trauma (5). These conditions result in a stable incidence of new patients who require long-term nutritional support. In North America roughly one person per two million population would be a candidate for bowel transplantation annually (excluding patients with inflammatory bowel disease) (6).

Short intestinal transplantation (SIT) as a treatment for SBS has been considered since the early twentieth century. At that time Carrell demonstrated the technical feasibility of such transplants, but commented that the rejection phenomenon would require definitive treatment before such operations could be considered further (7). As new therapies for immune suppression have developed, a flurry of experimental studies in SIT has ensued, with the occasional attempt at clinical use. Until recently, large animal studies and the experience in humans has been unfavorable. However, high dose monotherapy with cyclosporine A (CsA) has been shown to prevent rejection in outbred pigs undergoing SIT (8). Similar regimens have had limited success in attempts at SIT in humans (9-13). SIT, with combined liver transplantation has proven successful in six cases to date (13,14). It may be that combining SIT with either full or reduced size liver transplantation may be an extra step in immunomodulation that is required to permit SIT in man.

---

[1] A version of this chapter has been submitted for publication in the journal DIGESTIVE DISEASES.

Given these encouraging preliminary results, interest in SIT as a potential therapy for patients will increase. It is important to understand the limitations and potential problems that SIT as an alternate therapy to long-term TPN would have. This article will expand upon the previous reviews of techniques for SIT (15,16) emphasizing the experimental models used, the rejection process and our attempts to control it, and outline possibilities for future developments.

In reviewing these studies of bowel transplantation, it is important to bear in mind some of the variables of the experimental models used. Foremost of these is the interspecies variation in immune response following transplantation. Inbred strains of rats have a limited rejection response, while outbred strains of large animals such as pigs and dogs demonstrate much more vigorous rejection. Secondly, technical aspects such as the use of isolated Thiry-Villa loops, or the route of venous drainage of the engrafted bowel (caval or portal) may affect the immune response, and graft function (17). Finally, survival of a graft should not imply that it will be capable of absorbing nutrients.

EXPERIMENTAL TECHNIQUES AND MODELS

DOG

Transplantation of vascularized organs was first attempted by the French surgeon Alexis Carrell. His pioneering efforts at the turn of the century demonstrated the feasibility of SIT (7), using isolated loops of jejunum transplanted into the neck of dogs. Because of the problems of immune suppression, interest languished until the late 1950's, when Lillehei's group in Minnesota were investigating the effects of ischemia on gut organs (18). They found that cooling and perfusion with heparinized saline would reliably allow preservation of the small bowel for 4 hours, and that this preserved bowel could be reimplanted and would function indefinitely as an autograft (19). The model they developed consisted of a one-stage operation; the superior mesenteric vessels were isolated, clamped and divided, the bowel was flushed and then revascularized using the mesenteric vessels of a similarly prepared recipient, reestablishing bowel continuity using end-to-end anastomosis of the native duodenum and ileum to the graft. They also used isolated loops of bowel placed in the neck, permitting the study of immune suppressive agents and graft function in a controlled fashion; rejection of the graft would not lead to the death of the animal (19). They had no success with allografts. Other groups developed variations of these basic models, including shorter segments of bowel transplanted in or out of continuity with the gut using systemic venous drainage (20,21). Minimal survival advantage was found using the immune suppressants available at that time, azathioprine ALG and steroids (20-22).

The dog model was used to evaluate the rejection process in detail (see next section). After the introduction of CsA in the late 1970's, the orthotopic model of bowel transplantation in the dog was the first used to assess its effects on SIT (23,24). The significant prolongation of graft survival demonstrated by Reznick et al. in a landmark study was encouraging, but the overall rate of success was low; further details are discussed in the section on Immune Modulation Techniques.

Continued work with the dog model of SIT is reported, but the variability in the immunological differences between animals makes interpretation of survival data with immune suppression difficult (23,25,26). As well, differences in response to ischemia and reperfusion may limit it's applicability to studies in man (27,28).

RAT

The description of heterotopic bowel transplantation in the rat by Monchik and Russell in 1971 greatly facilitated study in this field (29). The rat model has since served as the standard for initial investigations of immune suppression, function, and techniques. In this model, the bowel is isolated using the aorta below the SMA and the portal vein as the vascular pedicle. It is then revascularized in the recipient using the infrarenal vena cava, and aorta. The bowel is left as a Thiry-Villa fistula with proximal and distal stomas (heterotopic graft). They also documented function of this bowel by resecting the native bowel and reestablishing gastrointestinal continuity using the transplanted segment (orthotopic graft). Kort described a one-stage procedure in 1973, wherein the native bowel was resected at the initial operation, with immediate reestablishment of gastrointestinal continuity (30). However, this was plagued with a high rate of technical failures (40%). Deltz described a two-stage procedure which had improved survival (80%) (31). At the same time, Lee and Schraut described their experience with the one-stage procedure; they had improved the technique described by Kort so that survival of isografts was greater that 80% (32). This then became the standard model for investigation of SIT in rats. The majority of these studies have been performed using a portal-caval anastomosis for venous drainage of the graft. However, Kort demonstrated the possibility of using a portal-portal anastomosis to provide a more physiological state posttransplant (30). This has been thought to confer some immunological advantages to the graft (17,30,33), and to establish a more physiological route for venous drainage from the graft (34). However, it remains a more difficult procedure, and so has not been widely used. More recently, other variations have been introduced, including combined small bowel and colon transplantation (35), the use of the renal pedicle for the vascular connections (36), and the use of the superior mesenteric artery as the arterial connection (37). These models have not been shown to offer any specific advantages over the previously described methods.

Within the rat model of SIT the availability of genetically defined strains of animals has greatly facilitated investigation of the immunological consequences of SIT (29,38). The availability of monoclonal antibodies to various cell populations in the rat should provide further valuable information.

PIG

The pig is an excellent model of human bowel physiology, with a more defined genetic lineage than the dog (39,40). Although earlier attempts had been made (41,42), Ricour and his colleagues were the first to successfully perform SIT in the pig (43) and achieve allograft survival. Subsequent studies have shown that although high levels are required, CsA monotherapy permits allograft survival and recipient growth following orthotopic SIT in the pig (8,43-46). The techniques used parallel those used in the dog, with both portal and caval routes of venous drainage being described. It is interesting that in those reports where allograft survival has been described, the graft was drained via the portal circulation of the recipient (8,43,45), while those reports where rejection occurred, caval drainage was employed (44,46).

IMMUNE RESPONSES FOLLOWING SMALL BOWEL TRANSPLANTATION: REJECTION AND GRAFT VERSUS HOST DISEASE

As noted in the introduction, the technical problems of SIT were solved by the early investigators. However, the problems of graft rejection and graft-versus-host-disease (GVHD) have remained formidable obstacles. The small bowel is unique amongst vascularized organ transplanted because the well-being of the recipient depends upon the continued integrity of the mucosal barrier. Rejection damages this barrier early, resulting in fluid losses, impaired nutrient absorption, and providing a portal of entry for enteric bacteria, in an already immune suppressed animal (47). Further, the transplanted bowel has a large population of immunocompetent cells, which can become activated following transplantation into a nonidentical recipient. This leads to a phenomenon known as GVHD, initially described following bone marrow transplantation. The factors which are necessary for significant disease development were summarized by Billingham: 1) the graft must contain immunologically competent cells; 2) the host must possess important transplantation isoantigens that are different from the donor, so that the host appears foreign to the graft; and, 3) the host itself must be incapable of mounting an effective immunological reaction against the graft for some period of time, allowing the graft to initiate a reaction against the host (48). This was recognized by Monchik and Russell in their initial studies of bowel transplantation in rats (29). They manipulated the reactions between the graft and host by using inbred strains of rats; tissue from the F1 progeny of a cross between two syngeneic strains (i.e. Brown Norway (BN) and Lewis (Lew) rats) into the parental strain allows for recognition of the graft as foreign (rejection or host-versus-graft, HVG), but not GVHD (LBN-F1→Lew). Conversely, transplantation of parental donor tissue into the F1 hybrid (Lew→LBN-F1) permits only the GVHD response. They demonstrated that the dominant response when both HVG and GVHD reactions are possible (BN→Lew, the two-way rejection response) is the HVG rejection, however this may have been a matter of timing, since the animals did not live long enough to develop classical GVHD.

They also described the histology of allograft rejection: at day 3, there were no changes, but at day 7 an infiltrate of lymphocytes in the mucosa was apparent, with edema, loss of villous height, and flattening of the epithelium. By day 14, there was complete loss of the normal villous architecture, and widespread inflammation and fibrosis. They noted that these findings were similar when only the rejection response was possible (LBN-F1→Lew) and when the two-way rejection and GVHD response was possible (BN→Lew). These observations were further refined by Rosemurgy and Schraut using a one-way rejection model (LBN-F1→Lew) (49). They further divided the rejection response into three phases. Phase 1 at day 6 and 7, lymphocytes and plasma cells begin to infiltrate the lamina propria. Phase 2 over day 8 and 9, the infiltrate intensifies, and extends to the muscularis propria. There is associated shortening and blunting of the villi and scattered epithelial sloughing. Phase 3, which occurs after the tenth day, there is complete mucosal destruction, and transmural infiltration with lymphocytes and polymorphonuclear leukocytes. Similar changes were observed in a two-way rejection model (Lew→BN), and significantly, these were not altered by low dose CsA treatment (10 mg/kg/day, given orally) (50).

Previous workers, studying the rejection process in untreated dogs, had described a similar process, but with a more rapid onset at 4 days posttransplant, with complete mucosal sloughing by 7 days (18,20,21,51). High dose CsA treatment alters this drastically; the mucosa is spared, and a lymphoplasmacytic infiltrate develops in the nerves and vessels of the submucosa and muscularis (52). This occurs in a progressive fashion, with an endpoint of graft fibrosis and loss of function, with death of the recipient. This lesion is histologically distinct from GVHD, and does not occur in the native bowel (25,52,53). The histopathology of acute rejection in pigs is similar, but chronic infiltrations of the submucosa and muscularis has not been not noted (8,42-46).

In the clinical setting a reliable and sensitive method to monitor for rejection is important. The nonspecific nature of the early stages of acute rejection noted above were found to limit the utility of suction biopsy in monitoring for rejection in the one clinical case that has been well documented (11). This, coupled with the possibility that rejection may cause significant changes in the deep mucosa have prompted investigators to search for alternate methods of monitoring for rejection (50,54). The functional capacity of bowel has been exploited as a marker by various investigators (22,55). The best characterized of these markers is the maltose absorption test described by Billiar et al. (56). This assay measures the ability of the bowel mucosa to split maltose into glucose and then transport this across the enterocyte into the circulation, where it is detected by monitoring serum glucose levels. The resulting rise is diminished when rejection is at its initial stages. Similarly useful predictions of allograft rejection are documented by measuring the permeability of the bowel. The most useful test of this type uses ^{51}Cr-EDTA instilled into the lumen of the graft. The bowel is normally impervious to this compound; an increase in absorption occurs with early graft rejection (57). This test has been used clinically and was found to correlate well with biopsy evidence of rejection (14).

A number of other possible markers of early allograft rejection have been suggested, including leakage of polyethylene glycol across the gut wall (58), changes in the ultrastructure of mucosa on biopsy (59), and alterations of gut hormone levels (60). None of these alternative strategies have been shown to have an advantage which justifies their increased complexity.

Aside from rejection, the transplant recipient may develop GVHD as described above. The characteristics of this disease are well described in the rat model of heterotopic

SIT (29,61-64). In one-way models where only GVHD can occur (Lew→LBN-F1) the animals are well until the ninth day posttransplant (62,63). Redness and swelling of the ears, nose, and paws are seen initially. They then develop dry, scaly skin, with alopecia, diarrhea, and lose weight rapidly, dying on the fourteenth postoperative day. The animals have marked loss of intraabdominal fat, hepatosplenomegaly, and marked destruction of residual native bowel, with sparing of the transplanted graft. T-cells must be present in the graft for GVHD to develop (38,65,66). These are the relevant "immunocompetent" cells of the graft described by Billingham (48). It has been demonstrated that donor lymphocytes move rapidly out of the transplanted bowel, and can be detected in the peripheral blood of the recipient within hours of transplantation (14,67,68). By 21 days posttransplant, lymphoid cells within the bowel are mostly of recipient origin, with a normal proportion of T- and B-cells (67).

GVHD can be prevented by various strategies which attack the population of immunocompetent cells of the graft, such as irradiation of the graft prior to transplantation (1000 rads ex vivo) (61,62,64), or by pretreatment of the donor with antilymphocyte serum (62,66,69,70). CsA (15 mg/kg/day orally for 14 days) or resection of the lymph nodes of the graft mesentery will allow 71% and 100% survival respectively (61,71) in this same model.

The requirement for immune suppression of the recipient for GVHD development has been demonstrated (72). Other strategies for preventing GVHD are discussed under the section on immune suppression techniques.

It is important to recognize that the GVHD seen in this experimental one-way model in inbred rats is not necessarily relevant in the clinical situation where both rejection and GVHD occur, and continuous recipient immune suppression is required. GVHD is not a lethal problem in any model of orthotopic SIT where both rejection and GVHD can occur (8,32,53,69). Using the two-way model in the rat and treating the recipient with a 1 week course of CsA posttransplant, GVHD causes a more mild disease than is seen with one-way models where only GVHD reaction can occur. Animals develop a non-lethal transient loss of weight, with paw and ear erythema 4-6 weeks posttransplant (69). However, using the one-way GVHD model and a heterotopic graft, immune suppression to at least 45 days is required to prevent lethal GVHD (38). In large animal models of SIT (both orthotopic, and heterotopic grafts), with and without immune suppression (8,45,52) there have been no problems which can be shown to be due to classical GVHD, although some workers have reported what they consider to be a blunted version of GVHD in dogs (53,73). In the limited clinical experience reported to date this has not been a significant problem either (12-14). Indeed, the immune suppression that GVHD produces must be considered after SIT (74); over immune suppression can set the stage for the development of lymphoproliferative disorders (75-77).

In summary, GVHD can cause the death of the recipient after SIT in models where one-way GVHD without rejection is the only immune response possible, but in situations where a two-way response is possible, it has not been a major problem.

IMMUNE SUPPRESSION: EXPERIMENTAL STUDIES

After the description of the techniques required for successful SIT in dogs, the principle factor limiting its use in clinical practise became the immune suppression required (19). Throughout the 1960's and 1970's, a number of different agents and manipulations were tried with little success. These were primarily attempts at controlling rejection using immune suppressive drugs posttransplant. Preston's group found that short segments (<20 cm) of jejunum would survive for up to 200 days in continuity with the gastrointestinal tract if treated with prednisone and azathioprine (20). Taylor showed that azathioprine would similarly prolong the survival of allografts placed in the neck, to 30 days (21). Hardy demonstrated that antilymphocyte serum with azathioprine and prednisone allowed graft survival to 38 days (22). Cohen showed that a low dose of radiation (50 rads, given ex vivo after the bowel had been harvested) prolonged allograft survival to 28 days (73). In each of these studies, the eventual fate of the graft was fibrosis, and loss of all apparent function.

The first important breakthrough for immunomodulation was the use of CsA to control rejection posttransplant (23). The average survival in 11 dogs treated with CsA (25 mg/kg/day, intramuscularly for the first 28 days, and then orally) was 91 days, while untreated controls survived an average of 12.5 days. However, it is important to note that only three dogs survived more than 60 days and two of these succumbed to rejection at 210 days. The importance of using parenteral CsA was demonstrated in a follow-up study from this group where a third set of animals was given CsA orally: seven of ten died of acute rejection at an average of 30 days posttransplant (24). The use of combined CsA and steroids has not significantly improved these results (25,26).

Once it had been shown that CsA prolonged the survival of intestinal allografts in dogs, a series of studies in the more controlled rat model appeared. It was shown that a dose of CsA of 15 mg/kg/day x6 days, then alternate days until day 28 allowed indefinite survival of grafts in unidirectional rejection and two-way rejection and GVHD models (32,78). As noted above in the section on GVHD, a similar dose of CsA will also control the GVHD occurring after transplantation (61,78). Strategies which pretreat the donor or host to prevent GVHD (64,66,70), or the use of a one-way model permitting rejection only (61,69) allows for the successful control of allograft rejection by a short course (7 days) of lower doses of CsA (5 mg/kg). However, if GVHD can occur, immune suppression to 28 days is required to prevent the development of GVHD with orthotopic grafts (35), while if the graft is in a heterotopic location, continued, higher dose CsA is required (38). Undoubtedly, GVHD plays some role in the ongoing requirement for immune suppression in these models.

The model of bowel transplantation available which most closely resembling the situation in man is the pig. Monotherapy with CsA has allowed successful transplantation in this model (8,43), however high doses are required (44,45). Grant has described a protocol of intravenous CsA (15 mg/kg/day) for 10 days posttransplant, and then 30 mg/kg/day orally (8). Lower doses of CsA, with segmental intestinal transplants have been used, but are not as reliable (45). Combined steroid and CsA use increased the rate of infectious complications, and did not reduce the rate of rejection (8). It is interesting to note that stopping CsA after roughly 3 months of continuous therapy did not lead to allograft rejection (8). The recipient may develop tolerance to the bowel allograft in a similar fashion to that described for liver transplants (79).

Other drugs which control rejection (and GVHD) posttransplant are being developed. FK-506 is a potent immune suppressant agent which has been shown to prolong survival of heterotopic (80) and orthotopically (81) transplanted bowel, and may be superior to CsA. 15-deoxyspergualin has also been shown to be adequate immune suppression for heterotopic graft survival (82). A number of more novel immune suppressants such as rapamycin and RS-61443 are currently being evaluated (83,84), which may prove useful in SIT, either as single agents, or in combination with others.

An alternative approach to controlling rejection is graft pretreatment. As discussed under the section of GVHD, pretreatment can diminish the GVH responses, and reduce the long-term immune suppression required. The various strategies employed (radiation, mesenteric lymphadenectomy, ALG, and monoclonal antibody) appear to be equally effective (61,64,69-72). It is likely that their beneficial effect is mediated by reducing GVHD, rather than affecting the rejection of the graft itself. There is little evidence to support this approach in large animals or humans. Radiation did prolong graft survival in a dog model, without other immune suppression (73), but was not effective in a pig model when used in

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An alternative approach to reducing rejection is to reduce the length of the graft (88,89). Kimura has shown in one-way (rejection or GVHD) rat models that with shortened transplanted segments (<30 cm) GVHD is not lethal, while with longer segments GVHD becomes increasingly problematic. The intensity of rejection, however, was relatively constant. This allowed survival of animals with shortened grafts using lower doses of CsA (88). This approach has been examined using a pig model, and has been partially successful with some survivors (3/11) using low doses of CsA (10 mg/kg/day intramuscularly) (45).

Liver transplantation has long been known to have a profound effect on rejection of other grafts (90-92). The nature of this phenomenon is still poorly understood, but may relate to non-self antigen processing by the transplanted liver or the release of MHC antigens in a soluble form, which then block cytotoxic antibodies (92). In clinical transplantation, improved survival of renal grafts following liver transplantation has been clearly documented, even in the presence of preformed antibodies to the graft (93).

At present, there is limited experimental work which has examined the survival of bowel after combined liver SIT (76,94). The one detailed study reported in rats used a cluster model, which included the stomach, pancreas, and colon with the liver and small bowel. No survival advantage was found for the bowel, when compared to isolated SIT, and death of the animals was due to rejection of the small bowel. A preliminary report dealing with a similar model in pigs, using oral CsA, had only one of twenty-two animals survive long-term (76). More specific studies are needed to examine the effects of combining liver and SIT, especially in larger animals.

SMALL INTESTINAL TRANSPLANTATION: CLINICAL EXPERIENCE

All attempts at SIT in man prior to the introduction of CsA were unsuccessful (95-97). The longest survivor lived for 76 days; even with an HLA identical donor and (delayed) treatment with steroids, azathioprine and antilymphocyte globulin, rejection and overwhelming sepsis from enteric flora proved fatal (95). Following the success of SIT using CsA in dogs and pigs, a number of attempts at human transplantation using CsA have been reported (9,10-12,98). The results have been poor. Typically, rejection is seen in graft biopsies on the fourth to tenth day posttransplant. Bowel fluid losses increase tremendously. Steroids and ALG have diminished the rejection response (10,11), but usually rejection has progressed necessitating graft removal around the fifteenth day posttransplant. Pretreatment of the graft with OKT3 antibody has not reduced the apparent immunogenicity of the bowel (11,12). Rejection has occurred despite portal drainage of the graft, but most patients requiring bowel transplantation have either a thrombosed portal vein, or multiple previous operations, prohibiting an anastomosis to the portal circulation (11,12,16). CsA regimens reported have included intermittent and continuous infusions (9-12), with levels of CsA that are therapeutic for kidney and liver transplants (200 to 400 ng/mL by whole blood monoclonal RIA) (99). Toxicity from immune suppression has been a common problem; CsA has resulted in renal toxicity (10), while combined therapy (steroid, ALG, azathioprine) has resulted in infectious complications (9-11). Children receiving bowel as part of a graft of multiple viscera have developed lymphoproliferative disorders (76,77). Clearly, these case reports demonstrate that with the current status of immune therapy, isolated SIT in man will remain difficult to perform. If a very close HLA match is obtained, rejection may be easier to control, however this has not been confirmed experimentally (95).

The evolution of the "cluster" or multivisceral transplantation grew out of the anatomical relationships of the abdominal viscera; rather than an attempt to manipulate the rejection process (76,77). However, the observation that combined immune suppression with CsA, prednisone, and ALG allowed prolonged survival of the associated bowel was important. It was logical to extend this to include a combined small bowel and liver graft for SBS with associated antithrombin III deficiency as described by Grant et al. (14). As reported by this group, a typical immune suppression protocol for isolated liver transplantation (OKT3, CsA, AZT and prednisone, with OKT3 pretreatment of the donor) has controlled rejection and GVHD (14). As described in the previous section regarding experimental immune suppression, there is no direct experimental work to validate this approach, however the good results of combining liver and bowel transplantation (3/3 patients survived with no episodes of graft threatening rejection) must be contrasted with the poor results of isolated bowel transplants (4/17 grafts functioning, with four deaths due to rejection complications) (9,11,12). Whether or not to include an associated liver graft from a patient with only SBS, and no primary liver pathology remains to be seen. Continued improvements in survival after liver transplantation (over 90% in nonemergent cases) (100) make such an approach a consideration. This would increase the demand for liver donors, however, retransplantation of the liver from the recipient of a combined liver/small bowel graft (the "domino" technique, as used in present heart/lung and heart transplantation) is theoretically possible. Selected patients, with life-threatening complications of SBS could ethically be considered for such therapy. Given the complexities of both the technical and immunological aspects of the procedure, the preliminary work should be concentrated in the centers with clinical and experimental experience in this area. Ongoing evaluation of the results will permit an accurate assessment of the risk/benefit ratio of this procedure (101).

FUNCTION OF TRANSPLANTED INTESTINE: AUTOGRAFTS AND SYNGENEIC GRAFTS

Lillehei and his coworkers showed in the initial investigations of SIT using dogs that animals could survive indefinitely following autotransplantation (18). No specific studies of nutrient absorption were performed, but they did note that gross malabsorption of fat was evident for 2-3 weeks, but then subsided. They were also able to demonstrate regeneration of the severed lymphatics after 3 weeks (102), this did not occur in allografted dogs not receiving immune suppression probably because they did not live long enough (103).

Ballinger et al. performed more detailed studies of gastrointestinal function following autotransplantation in dogs (104). They demonstrated that following transplantation of the small bowel dogs had a period of 2-3 weeks of diarrhea, weight loss, and abnormal motility. Fat absorption was reduced to 40% (normal 88-90%). These changes reversed over the ensuing months and had normalized by 6 months. A very similar pattern of changes was produced by denervating the bowel and dividing the lymphatics; they concluded that the functional alterations they observed after transplantation were from the denervation and lymphatic disruption, and that they were reversible.

Using a different model of an isolated Thiry-Villa fistula of autotransplanted bowel, Sarr and Kelly have demonstrated that the transplanted bowel can develop an interdigestive myoelectric complex, but that these are not normally controlled since they were not suppressed by feeding, or temporally related to the electrical activity of the intact bowel (105).

A possible relationship between these changes in neural activity and the function of the bowel was demonstrated by Watson et al. (106). They showed in the rat that heterotopic isografts and denervated Thiry-Villa fistulas had reduced absorption of glucose, glycine, water, and electrolytes. Chloride was most significantly affected, with net secretion in some instances. They noted that the crypts are under greater autonomic nervous control than the villi, and that the crypts tend to secrete chloride while the villi absorb it. They postulated that transplantation, or the denervation process may allow for continued hypersecretion, due to a loss of tonic inhibition of the crypts by the autonomic nervous system (107). A similar study performed in dogs failed to demonstrate such changes (108,109); further studies will be required to determine if this pattern of altered function with denervation occurs in larger animals.

Short-term evaluations of the electrophysiological parameters of the bowel (performed at 9 days posttransplant) showed that spontaneous potential difference, and resistance were unaffected by transplantation itself (38,110). Overall, these findings suggest that the transplantation process itself, independent of rejection and ischemic injury, significantly affects the motility, neural, and transport functions of small bowel. The foregoing studies all were performed on isolated loops of transplanted bowel. In the few studies that have been reported on orthotopic, syngeneic or autografted bowel, significant reductions in nutrient absorption have been described. This is most pronounced for fat, but was also noted for carbohydrate, up to 11 months posttransplantation in dogs (111).

INTESTINAL FUNCTION: ALLOGRAFTS

Prior to the introduction of CsA, several limited studies were reported examining the function of allografted bowel. Holmes et al. demonstrated that transplanted bowel was capable of absorbing glucose, but that radiolabelled glucose uptake was a poor marker of intestinal function and rejection (55). The leakiness of the bowel wall induced by rejection resulted in the nonspecific diffusion of glucose into the systemic circulation, however the specificity of this as a marker for rejection was improved by using lower concentrations of glucose (112). Tracer studies also demonstrated that both allo- and autografts in dogs would absorb short-chain fatty acids (lauric) soon after transplantation (day 2), but long-chain fatty acids (oleic) required regeneration of lymphatics before significant absorption occurred (113). Ruiz et al. showed that allografts could absorb d-xylose, and vitamin A, albeit poorly, with maximal absorption of xylose being 40% of normals (114).

A number of studies have investigated the function of allografts treated with CsA. Beginning at the enterocyte level, Kirkman showed that the rejection process reduces the transepithelial potential difference of the bowel, and reduces the resistance (38,110). These effects were partially, but not completely ameliorated by CsA (15 mg/kg). The function of brush border membrane enzymes (peptidases and disaccharidases) appears to be normal so long as rejection does not occur. However the studies that have been done investigating this have not been well controlled (115).

A series of investigations have been performed examining the absorptive function of allografted loops of bowel in the rat (using the heterotopic model). Glucose, glycine, and electrolytes are all absorbed normally so long as rejection is not occurring. However, with the earliest signs of rejection morphologically, function deteriorates (106,116). In the dog model of heterotopically transplanted bowel, there was a persistent loss of protein from allografted bowel, which resulted in profound hypoproteinemia (109). So far as could be determined, glucose, glycine, and electrolyte transport was similar to that seen in autografted bowel (109).

There have been even fewer studies of the functional capabilities of orthotopic allogeneic SIT. In the rat model, allografted animals have been shown to have low serum triglycerides, and vitamin A levels, with high fecal fat losses a year following transplantation, despite apparent good health and growth (117). No detailed studies of nutrient absorption and electrophysiological function of transplanted bowel which is actually supporting the animal's nutrition have been reported, in any model. In large animal models, long surviving pigs have been shown to have normal serum lipid profiles, normal xylose absorption, and normal levels of fecal fat (8), but actual nutrient absorption has not been quantified further. These questions are important, because the length of bowel required to sustain the recipient posttransplant is a critical parameter. Given the present trials of bowel transplantation in humans, this requires further investigation.

CYCLOSPORINE: EFFECTS ON INTESTINAL FUNCTION

To date, only limited studies of the effects of CsA on bowel function have been published (118,119). However, there is evidence that CsA may affect nutrient absorption. Transplant patients of all types receiving CsA have been noted to have occasional episodes of profound diarrhea, which is usually attributed to the olive oil vehicle of CsA. Collin et al., in their investigation of the function of dog allografts of small bowel, noted that autografted bowel in animals receiving CsA had diminished glucose, alanine, and lauric acid absorption (109). Sigalet et al. have shown that moderate doses of CsA (15 mg/kg/2 days, subcutaneously) do not affect weight gain or nutrient absorption in the rat, but do reduce glucose uptake in vitro (118). Higher doses (30 mg/kg) given orally or subcutaneously have similar effects in vitro, with reduced weight gain and nutrient absorption from the diet (119). These changes may occur from a direct effect of CsA on the sodium-glucose cotransporter of the bowel epithelial cells, as has been described in the kidney (120). Further studies are necessary to clarify the mechanisms underlying these observations, however it is clear that CsA does have an effect on bowel function which may be significant when used in patients with impaired gastrointestinal function.

In summary, the introduction of CsA as an immune suppressant has allowed for controlled studies of allogeneic SIT. A variety of models have demonstrated the feasibility of such transplants in allowing continued survival and growth of the recipient. Preliminary successes with human small bowel transplant has been achieved, but many questions remain regarding graft function, and the optimal strategies for preventing rejection. As we learn more about transplant immunology we will be able to design more specific strategies, which will allow graft survival while minimizing toxicity to the host (121). The present level of activity in this area will only increase as we improve our techniques.

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CHAPTER III

CYCLOSPORINE EFFECTS ON NORMAL BOWEL[1]

INTRODUCTION AND METHODS

Cyclosporine (CsA) has greatly expanded the horizons of transplantation and has renewed interest in clinical small bowel transplantation (SIT) (1). CsA is also being used in the treatment of autoimmune diseases and is being evaluated as a treatment for inflammatory bowel disease. However, CsA has a range of adverse effects on the function of various grafts and host tissues. These include such problems as nephrotoxicity, impairment of insulin release by islets, and effects at the cellular level on sodium/glucose transport (2,3). Because of these known effects, we sought to determine the effects of CsA on bowel function in normal rats as a prelude to studies involving transplanted bowel.

Female Lewis rats (200-220 g) were obtained from Charles River Canada (St. Constant, PQ), and were treated with either CsA (Sandimmune, Sandoz Pharmaceutical Corp., Montreal, PQ), or control solvent. A typical SIT immune suppression protocol was used, with animals receiving subcutaneous injections of CsA (10 mg/mL dissolved in medium chain triglyceride oil, dosage: 15 mg/kg) or an equivalent volume of control oil daily for 3 days, and then injections on alternate days for 60 days (4). There were ten animals in the control group and thirteen in the CsA treated group. Animals were individually housed in Plexiglas® cages, allowed free access to feed (Tekland Premium Lab Diet, Textron Corp., Madison, WI) and water, and monitored with weekly determinations of feed intake and weight.

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[1] A version of this chapter has been accepted for publication in the journal TRANSPLANTATION.

The nutritional effect of CsA was assessed over the final week of treatment by performing a balance study to quantify nutrient absorption. Animals were preconditioned in metabolic cages, and a 3 day balance study was carried out, with daily quantitative fecal collections. Total energy, fat, protein, and carbohydrate content of feed and feces were determined using standard methods (5), and nutrient absorption calculated directly. After 60 days of treatment, animals were sacrificed and in vitro studies of nutrient uptake were performed using methods detailed elsewhere (6,7).

In vitro glucose uptake was assessed as follows. Animals were anesthetized with pentobarbital and short segments of bowel were rapidly removed, rinsed with 50 mL of cold saline, opened along the mesenteric border, and the mucosal surface carefully washed with a stream of cold saline to remove visible mucus and debris. The intestine was cut open, mounted as flat sheets in incubation chambers, and clamped between two plastic plates so that the mucosal and serosal surfaces were exposed to separate incubation solutions, with apertures in the plates exactly 1.0 cm in diameter.

The solutions were mixed at identical stirring rates with circular magnetic bars, with stirring rates precisely adjusted by means of a strobe light. In these studies, the stirring rates were set at 600 rpm to yield low values of the effective resistance of the intestinal unstirred water layer. After preincubation in Krebs-bicarbonate buffer for 15 minutes, the transport chambers were transferred to other beakers containing [^{3}H]-inulin and various probes including [^{14}C]-labelled D-glucose, L-glucose, cholesterol, and fatty acids in oxygenated Krebs-bicarbonate buffer at 37°C and pH 7.2. The concentration of D-glucose was varied from 1-64 mM, and the concentration of L-glucose was 16 mM. The fatty acids included: stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3). The long-chain fatty acids (18:0 to 18:3) were prepared in a concentration of 0.1 mM, and were solubilized in

20 mM taurodeoxycholic acid (TDC). The cholesterol was prepared in a concentration of 0.05 mM in 20 mM TDC (6).

After incubation for 6 minutes in one of these test solutions, the experiment was terminated by removing the transport chamber and quickly rinsing the intestinal tissue in cold saline for approximately 5 seconds. The exposed mucosal tissue was then cut out of the chamber with a circular steel punch, placed on glass slides, and dried overnight to a constant weight in an oven at 55°C. The dry weight was determined and the tissue was transferred to scintillation counting vials. The sample was then saponified with 0.75 N NaOH, scintillation fluid was added and radioactivity was determined by means of an external standardization technique to correct for variable quenching of the ^{3}H- and ^{14}C-isotopes. CsA levels were determined at sacrifice, using whole blood CsA specific monoclonal antibody assay (Cyclo-Trac SP ^{125}I RIA Kit, Incstar Corp., Stillwater, MN). Complete blood counts were performed with a Coulter counter (M4-30, Coulter Electronics, Hialeah, FL), and creatinine levels were determined using a multistat analyzer (IL-Multistat III, Instrumentation Laboratories, Lexington, MA).

The kinetic characteristics of D-glucose transport were determined by plotting uptake versus concentration. Active transport was defined as the observed uptake of D-glucose less the uptake of L-glucose at the same concentration. Apparent Michaelis constant (Km*) and jejunal maximal transport rate (Vmax) values and their associated confidence limits were derived from the resulting curves using a least squares fit to determine the best line using a statistical package (8). The remaining data was compared using Student's t-test (two-tailed), with a p value of <0.05 taken as being significant. All results are expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

CsA treatment did not affect the growth of the animals or the in vivo absorption of nutrients from the diet (Table III-1). However, active glucose transport and the passive uptake of stearic (18:0) and linolenic (18:3) fatty acids were reduced in the jejunum of CsA-treated animals (Tables III-1, III-2). These animals also had a decline in the value of the Vmax for D-glucose without a change in the Km*. In the ileum, CsA treatment increased the Vmax of glucose transport, with no effect on fatty acid uptake (Table III-1).

CsA levels were within the therapeutic range for immune suppression (Table III-1). CsA had only a slight effect on intestinal morphology, increasing the dry weight of the mucosa per cm^2 serosa in the ileum (7.0±0.3 in CsA-treated animals versus 5.6±0.6 mg/cm^2 in controls, $p \leq 0.05$), but did not change bowel length, diameter, or mucosal weight in jejunum (data not shown).

The results of the in vitro uptake studies in the control group are similar to those found in other studies of normal rats (9), while the findings in the CsA-treated animals suggest that CsA affects the sodium-glucose cotransporter in the bowel. These changes are subtle, but were consistent in treated animals. Further studies will be required to determine if similar effects occur at different doses and with different routes of administration.

Reduced activity of the sodium-glucose cotransport carrier have been described in kidney tubule cell culture lines treated with CsA (3). A reduction in the activity of this pump, the dominant transport mechanism for glucose in the bowel would explain the reduction in glucose transport observed in the jejunum of the treated animals. The increase in the Vmax for glucose seen in the ileum could be compensatory; a result of an increase in glucose load in the ileum due to decreased uptake of glucose in the proximal intestine. A less likely possibility is that CsA has a different direct effect on the sodium-glucose carrier in the distal as compared to the proximal intestine.

The observed effects on fatty acid uptake are less straightforward. CsA is not known to have an effect on membrane composition (2), but does decrease both bile salt and bile fluid output in the rat (10). The selective decrease in 18:0 and 18:3 uptake in the jejunum of CsA-treated animals may reflect alterations in fatty acid metabolism brought about by the decrease in bile output, or may be a result of previously unrecognized changes in the cell membrane of the enterocytes. It is unlikely that CsA alters the dimensions of the intestinal unstirred water layer, since the magnitude of the Km* was unchanged.

The mechanisms underlying these observations are unknown at present, but four main possibilities exist. CsA may directly affect the activity of the Na-glucose cotransporter. No previous studies have examined the effects of CsA on transport across intact epithelium in any organ. The changes observed in this study may occur in other tissues, and could explain some of the effects of CsA on other organs, such as the kidney. Secondly, in bowel CsA may reduce blood flow through the capillary bed of the villi in a manner similar to that described in the renal glomerulus (11). Such changes in blood flow could secondarily affect cellular transport capabilities but are unlikely to affect nutrient transport measured in vitro. Thirdly, CsA may be acting on the enterocyte membrane, either directly or via alterations in bile flow. Such membrane changes would explain the observed alterations in passive fatty acid uptake, as well as the alterations in glucose transport. Finally, CsA's primary therapeutic effect is to alter the function of t-"helper" lymphocytes. These may interact with the enterocyte and influence nutrient transport. The interactions between the gut and the immune system are largely unknown (12). Further studies are necessary to examine these various possibilities.

The observations that CsA treatment affects glucose and fatty acid uptake in normal bowel has implications for its future use in immune suppression generally, and specifically for bowel transplantation. The animals in this study had normal bowel, and although no changes were seen either in animal growth or nutrient absorption, in situations where bowel length or functional capacity is reduced (small intestinal transplantation, inflammatory bowel disease), the effects seen in vitro in this study could be deleterious to the overall well-being of the transplant recipient.

TABLE III-1: In vivo and in vitro effects of cyclosporine on intestinal function in the rat.

	Controls	CsA Treated
ANIMAL CHARACTERISTICS		
Final Weight (g)	251±6	252±7
Weight gain (g)	56±8	54±7
Feed Intake (g/day)	14±1	13±2
NUTRIENT ABSORPTION IN VIVO*		
Total Energy	84±1	84±1
Protein	76±1	77±2
Fat	79±1	80±2
Carbohydrate	93±2	93±2
CYCLOSPORINE LEVELS		
Peak (μg/mL)	0	368±44
Trough (μg/mL)	0	311±96
NUTRIENT UPTAKE IN VITRO		
Active glucose uptake		
Jejunum		
Vmax	622±99	538±106†
Km*	20±6	14±5
Ileum		
Vmax	504±80	636±144†
Km*	3±2	11±6

Results expressed as mean±SD

* % of nutrient absorbed by the animal

† $p<0.05$

Vmax (maximal transport rate) has the units nmol·100 mg mucosa^{-1}·min^{-1}

Km* (apparent affinity constant) has the units mM.

TABLE III-2: Effect of cyclosporine on lipid uptake.

	JEJUNUM		ILEUM	
	Control	Cyclosporine	Control	Cyclosporine
18:0	0.90±0.16	0.41±0.10*	0.60±0.13	0.75±0.16
18:1	0.70±0.10	0.59±0.06	0.88±0.13	0.83±0.14
18:2	0.69±0.11	0.60±0.12	0.65±0.13	0.81±0.12
18:3	0.91±0.10	0.57±0.13*	0.57±0.08	0.71±0.15
Cholesterol	0.93±0.09	1.29±0.17	2.13±0.39	2.97±0.61

Results expressed as mean±SD.
Units are nmol·100 mg mucosa^{-1}·min^{-1}
* $p<0.05$, cyclosporine versus control

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CHAPTER IV

CYCLOSPORINE REDUCES NUTRIENT ABSORPTION IN NORMAL RATS[1]

INTRODUCTION

Protocols for immune suppression following transplantation now almost universally use cyclosporine (CsA). Although this is still the largest population of patients treated with this drug (1), CsA's unique activity has stimulated interest in a large number of other possible clinical indications. These range from control of autoimmune diseases such as uveitis (2), type I diabetes (3), nephropathy (4), and idiopathic inflammatory bowel disease (5). In the transplant population, gastrointestinal side effects such as nausea, bloating, and diarrhea are commonly seen with CsA use, and are often attributed to the oil solvent (1). However, the possibility of CsA activity in cells outside the immune system (6), and the description of effects of CsA on glucose transport in kidney tubule cells (7), initially led us to investigate the direct effects of CsA on nutrient uptake by the rat intestine. We found that CsA given following a typical protocol for transplantation in the rat (15 mg/kg subcutaneously on alternate days) caused a significantly reduced passive intestinal fatty acids (8). This prompted the present study, in which we examine the effects of varying the dose and route of administration of CsA on nutrient handling by the rat bowel. Our hypothesis is that CsA impairs glucose absorption (in a dose-dependent fashion), independent of route of administration.

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[1] A version of this chapter has been submitted for publication in the journal TRANSPLANTATION.

MATERIALS AND METHODS

ANIMALS

Male Lewis rats (250-270 g) were obtained from Charles River Canada (St. Constant, PQ). They were housed individually in Plexiglas® cages, with free access to feed (Tekland Premium Lab Diet, Textron Corp., Madison, WI) and water. They were treated with CsA given orally by gavage daily, or by subcutaneous injection (Table IV-1) for 30 days. CsA (Sandimmune, Sandoz Pharmaceutical Corp., Montreal, PQ) for injection was prepared by dissolving 5 or 30 mg/mL crystalline CsA in medium-chain triglyceride oil (Mead Johnson, Ottawa, ON), and sterilized by microfiltration. CsA for gavage was prepared by dissolving 3.25 or 15 mg/mL in MCT oil. Animals were monitored with weekly determination of feed intake and body weight.

The nutritional effect of CsA was assessed over the final week of treatment by performing an in vivo balance study to quantify nutrient absorption. Animals were preconditioned in metabolic cages, and a 3 day balance study was carried out, with daily quantitative fecal collections. Total energy, fat, protein, and carbohydrate content of feed and feces were determined using standard methods (9,10), and nutrient absorption was calculated directly. After 30 days of treatment, animals were sacrificed, and in vitro studies of nutrient uptake were performed using previously described methods (11,12), as outlined in brief below.

IN VITRO GLUCOSE UPTAKE

The animals were anesthetized with pentobarbital and short segments of bowel were rapidly removed, and rinsed with 100 mL iced oxygenated saline. The intestine was then split along the mesenteric border, and the mucosal surface was carefully washed with a stream of cold saline from a syringe to remove visible mucus and debris. Sections were then mounted, as flat sheets in incubation chambers, and clamped between two plastic plates so that the mucosal and serosal surfaces were exposed to separate incubation solutions, with apertures in the plates exactly 1.0 cm in diameter.

The preincubation and incubation solutions were mixed at identical stirring rates with circular magnetic bars, and the stirring rates were precisely adjusted by means of a strobe light. In these studies, the stirring rates were set at 600 rpm to yield low values of the effective resistance of the intestinal unstirred water layer.

After preincubation in Krebs-bicarbonate buffer for 15 minutes, the transport chambers were transferred to other beakers containing [^{3}H]-inulin and various probes including [^{14}C]-labelled D-glucose, L-glucose, cholesterol and fatty acids in oxygenated Krebs-bicarbonate buffer at 37°C and pH 7.2. The concentrations of D-glucose were varied from 1-64 mM, and the concentration of L-glucose was 16 mM. The fatty acids included: dodecanoic (12:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3). The long-chain fatty acids (18:0 to 18:3) were prepared in a concentration of 0.1 mM, and were solubilized in 20 mM taurodeoxycholic acid (TDC). The cholesterol was prepared in a concentration of 0.05 mM in 20 mM TDC (12).

After incubation of the jejunal tissue for 6 minutes in one of the test solutions, the experiment was terminated by removing the transport chamber and quickly rinsing the intestinal tissue in cold saline for approximately 5 seconds. The exposed mucosal tissue was then cut out of the chamber with a circular steel punch, placed on glass slides and dried overnight to a constant weight in an oven at 55°C. The dry weight was determined and the tissue was transferred to scintillation counting vials. The sample was then saponified with 0.75 N NaOH, scintillation fluid was added and radioactivity was determined by means of an external standardization technique to correct for variable quenching of the ^{3}H- and ^{14}C-isotopes.

At sacrifice jejunal and ileal tissue was taken, pinned on a cork board, and fixed in formalin. Morphology (villus height, width and mucosal surface area) was assessed using standardized methods (13).

STATISTICAL METHODS

The kinetic characteristics of D-glucose transport were determined by plotting uptake versus concentration. Active transport was defined as the observed uptake of D-glucose less the uptake of L-glucose at the same concentration (the passive uptake of L-glucose was shown to be linear over the range from 0 to 64 mM, data not shown). Apparent Michaelis constant (Km*) and maximal transport rate (Vmax) values and their associated confidence limits were derived from the resulting curves, using a least squares fit to determine the best line using a statistical package (14). The remaining data was compared using ANOVA, with a p value of ≤0.05 taken as being significant. All results are expressed as mean±SEM.

CsA levels were determined at sacrifice using whole blood CsA-specific monoclonal antibody assay (Cyclo-Trac SP ^{125}I RIA Kit, Incstar Corp, Stillwater, MN). Complete blood counts were performed with a Coulter counter (M4-30, Coulter Electronics, Hialeah, FL), and creatinine levels were determined using a multistat analyzer (IL-Multistat III, Instrumentation Laboratories, Lexington, MA).

RESULTS

High dose CsA significantly reduced the weight gain of animals, whether given orally or by subcutaneous injection (Table IV-2), without a significant change in feed intake. The process of gavaging animals daily did cause a significant reduction of weight gain and feed intake in itself. However, we have compared animals only within the same route of treatment category. Both high and low dose CsA given by gavage reduced dietary fat and energy absorption (Table IV-2). Energy absorption appeared to be affected in a dose dependent fashion, with the high dose group absorbing significantly less energy than the low dose group. There was no similar dose effect with fat absorption, but these animals were maintained on a low fat (4% of total calories) diet. Subcutaneously injected CsA did not affect nutrient absorption (fat or total energy) from the diet.

Nutrient uptake in vitro was significantly affected by CsA treatment (Tables IV-3, IV-4). Subcutaneously injected low dose (5 mg/kg) CsA significantly reduced both ileal and jejunal Vmax, while high dose CsA reduced the Km* for active glucose transport. Both these effects reduce the ability of the intestine to transport glucose. Both high and low dose oral CsA reduced ileal Vmax by 50%, without affecting the Km*. A similar trend was observed in the jejunum ($p<0.1$), but did not achieve statistical significance. It is interesting to note that the groups with the reduction in ileal Vmax were the ones which had reduced absorption of dietary total energy.

Subcutaneous administration of CsA, both low and high dose, reduced the uptake of the fatty acids 18:0, and 18:2, and cholesterol (Table IV-4). Oral CsA caused a similar trend in both the ileum and jejunum ($p<0.1$).

Microscopic morphology was affected by CsA. In the jejunum, high dose oral CsA (30 mg/kg) was associated with an increase in villus height (477±20 versus 394±17 μm CsA versus controls, $p<0.05$), but did not change the mucosal surface area (Table IV-5a). The other treatment groups did not show major changes in jejunal morphology. In the ileum, low dose oral CsA caused increases in villus size and number, with a net increase in mucosal surface area, while high dose oral CsA abolished these changes (Table IV-5b). Low dose subcutaneous CsA had the opposite affect: villus size was reduced, with a net reduction in mucosal surface area. High dose subcutaneous CsA again minimized these changes.

CsA levels were consistent with the doses given (Table IV-2). There were no changes in the hematological parameters measured (CBC, serum electrolytes, and creatinine) from CsA treatment (data not shown).

DISCUSSION

These results corroborate our previous findings of reduced in vitro glucose and fatty acid uptake with moderate doses (15 mg/kg subcutaneously alternate days) of CsA. We have extended these in vitro results to show that high dose CsA reduces weight gain and that oral CsA reduces dietary nutrient absorption. Previous investigators have demonstrated similar reductions in weight gain with high dose parenteral CsA (15,16).

In general, weight gain in a growing animal can be affected by feed intake, nutrient absorption, and other metabolic demands on the animal. Our studies have focussed on CsA's effects on nutrient absorption; in reviewing the process of nutrient digestion and subsequent absorption, a number of potential mechanisms for the observed effects of CsA are suggested (17,18). In these studies, CsA did not affect the amount of food ingested by animals (Table IV-2), and is not known to alter gastrointestinal motility patterns. While our studies did not examine the intraluminal phase of nutrient digestion, previous investigators have shown in the rat that bile flow (both bile salt dependent and independent flow) is reduced by acute and chronic administration of CsA (16,19,20). This in turn can reduce the emulsification of dietary fat, and its subsequent uptake. Bile flow can also secondarily affect intestinal morphology; reduced bile flow reduces villus size and surface area (21). The effects of CsA on the production and activity of intraluminal digestive enzymes in the adult rat are not known. In weanling rats, CsA causes a delay in intestinal maturation, and a reduction in disaccaridase activity (15). Accordingly, CsA could adversely affect the emulsification of dietary fat, and the intraluminal digestion of proteins and carbohydrates.

Our data suggests that CsA reduces nutrient absorption by reducing uptake by the enterocyte. All doses and routes of CsA administration affected active glucose uptake in vitro (Table IV-4). Previous authors have noted an effect in renal cell culture lines on sodium glucose cotransport (6); this carrier is the dominant pathway for active glucose uptake in the small intestine (17). In our studies, the effect of CsA on active glucose uptake does not appear to be straightforward: the reduction in glucose uptake was not dose dependent (although tissue levels of CsA were not measured), and CsA affected both Vmax and the Km for glucose. CsA may directly reduce the activity of this carrier, or it's affinity for glucose. There is no evidence available regarding CsA's direct effect on the activity of the sodium-potasium ATPase pump of the basolateral membrane, although other authors have postulated that decreased activity by this enzyme may explain the reduction in bile flow seen with CsA (19).

CsA may also affect the composition of the enterocyte membrane, which in turn can affect transmembrane enzyme activity, intracellular junctions, and transmembrane passive permeability (22). CsA is known to alter the phospholipid content of renal tubular cells (23). We did not examine enterocyte membrane composition directly, however given the widespread changes in fatty acid uptake observed (Table IV-4), CsA induced changes to enterocyte membrane composition are probable (18). Thus, CsA's known effects, and those described in the present study, suggest that CsA could adversly affect both nutrient digestion and subsequent transport across the intestinal epithelium. The variability in effects with different routes of administration was anticipated. CsA is metabolized by the intestine (24), and tissue levels vary greatly (25). Thus, blood levels of CsA do not reflect the ??? concentration in the intestinal mucosa.

CsA could also be affecting nutritional status in an indirect fashion, by increasing the metabolic demands on the animal. Such changes in metabolic activity occurred in this study;

the group receiving high dose CsA subcutaneously had a significant reduction in weight gain, without a reduction in dietary nutrient absorption. CsA's metabolic interactions are best understood in the kidney. There, CsA leads to renal tubular atrophy, which may be caused by an increase in intracellular protein degradation (26). If such effects occur in other tissues, they would become a significant metabolic load for the animal. Similarly, the reduced activity of the sodium-glucose cotransport system noted may cause a reduced efficiency of intestinal and renal transport systems, which in turn could increase energy requirements.

Secondary effects of CsA could also influence nutrient absorption. The renal toxicity of CsA is thought to be due changes in blood flow at the arteriolar level (27). Preliminary studies of the effect of CsA on the intestine have shown a reduction in vascularity, which is biphasic in nature (28). High doses show a normalization in blood vessel area, possibly due to neurotoxicity. We noted a similar biphasic pattern in the changes in in vitro glucose uptake and intestinal morphology.

Finally, CsA's main effect on the immune system is to reduce the activity of t-helper lymphocytes (1). The lamina propria of the bowel has an extensive population of such lymphocytes; while the relationship between the immunological status and absorptive capacity of the bowel is not well understood, there is evidence that changes in immune status can affect intestinal function (29) . The continued improvements in our understanding of the molecular mechanisms of CsA's effects on the immune system may help us to understand which of the foregoing mechanisms is responsible for the observed effects on nutritional status in these animals (1,23). With improved understanding of mechanisms, stratagies to minimize these effects can be devised (30).

The observation that CsA affects bowel function has important implications as the indications for CsA use increase. Patients with co-existing bowel dysfunction may note a deleterious effect on their nutritional status. Further studies are indicated to determine both the mechanism(s) underlying these observations, and their significance in man.

TABLE IV-1: Cyclosporine doses.

Route:	SUBCUTANEOUS			GAVAGE		
CsA Dose:	controls	5 mg/kg	30 mg/kg	control	7.5 mg/kg	30 mg/kg
Volume of Solution	0	1 mg/kg body wt*	1 mg/kg body wt*	2 mL†	2 mL†	2mL†

* alternate days

† daily

TABLE IV-2: Animal characteristics following CsA treatments.

Route:	SUBCUTANEOUS			GAVAGE		
Dose:	control	5 mg/kg	30 mg/kg	control	7.5 mg/kg	30 mg/kg
Weight gain (g/day, 0-30)	70±6	72±8	53±8‡	40±10‡	42±7	31±6*
Feed intake (g/day)	20±1.8	18±3	16±2	11±2‡	13±1.6	11±2.2
CsA levels (ug/mL)	0	240±40	1820±410	0	490±90	1350±100
NUTRIENT ABSORPTION (% NUTRIENT ABSORBED FROM DIET)						
Total energy	84±1	83±1	84±2	90±2‡	87±1*	83±2†
Fat	79±1	78±2	79±2	96±1‡	93±1*	92±2*†

(Note: comparisons made between dosages of CsA, within routes of treatment - see text)

‡ $p<0.05$ versus injected controls

* $p<0.05$ versus gavaged controls

† $p<0.05$ versus low dose gavage

TABLE IV-3: Cyclosporine effects on active glucose uptake in normal rats.

Route:	SUBCUTANEOUS			GAVAGE		
Dose:	control	5 mg/kg	30 mg/kg	control	7.5 mg/kg	30 mg/kg
JEJUNUM						
Vmax†	1226±128	792±194‡	1166±669	634±131	356±79	486±103
Km†	16.1±3.0	18.5±7.2	54.3±38.8‡	6.8±3.1	7.3±3.9	5.9±2.9
ILEUM						
Vmax†	588±64	383±85‡	721±405	981±324	495±103*	468±136*
Km†	9.3±0.7	3.8±2.0	20.3±16.0‡	21.5±9.8	12.9±5.1	17.9±10.3

† Vmax units = nmol·100 mg mucosa^{-1}·min^{-1}, Km = mM

‡ $p<0.05$ versus injected controls

* $p<0.05$ versus gavaged controls

TABLE IV-4: Cyclosporine effects on lipid uptake in normal rats.

	ORAL			SUBCUTANEOUS INJECTION		
	Control	7.5 mg/kg	30 mg/kg	Control	5 mg/kg	30 mg/kg
JEJUNUM						
12:0	6.32±2.04	14.64±5.93	13.40±4.09	NA±NA	17.40±3.57	11.63±1.47
18:0	0.6±0.25	0.21±0.21	0.38±0.17*	0.90±0.16‡	0.32±0.04†	0.46±0.10†
18:1	0.81±0.39	1.11±0.18	0.40±0.13	0.70±0.10	0.58±0.10	0.57±0.11
18:2	0.81±0.27	0.55±0.20	0.60±0.21	0.69±0.11	0.95±0.18	0.84±0.16
18:3	0.40±0.26	0.49±0.16	0.74±0.22	0.91±0.10	0.80±0.16	0.55±0.07
Cholesterol	0.41±0.15	0.32±0.08	0.23±0.05	0.93±0.09‡	0.44±0.09†	0.60±0.12†
ILEUM						
12:0	12.83±1.93	7.48±2.16	20.85±2.70*	NA	17.10±3.23	11.16±1.55
18:0	0.32±0.06	0.17±0.07	0.05±0.01*	0.60±0.13	0.51±0.08	0.41±0.07†
18:1	0.23±0.07	0.41±0.09	0.18±0.08	0.88±0.13‡	0.49±0.10	0.44±0.12
18:2	0.70±0.17	0.49±0.14	0.16±0.05	0.64±0.13	0.89±0.12	0.54±0.11
18:3	0.62±0.12	0.39±0.15	0.19±0.08	0.57±0.08	0.57±0.09	0.41±0.08
Cholesterol	0.35±0.07	0.29±0.08	0.21±0.04	2.13±0.39‡	0.42±0.09†	0.35±0.06†

* $p<0.05$, oral cyclosporine versus control

† $p<0.05$, injected cyclosporine versus control

‡ $p<0.05$, injected versus oral controls

TABLE IV-5: Cyclosporine effects on intestinal morphology in normal rats.

TABLE IV-5A. JEJUNAL MORPHOLOGY

	ORAL			SUBCUTANEOUS INJECTION		
Morphological Parameter	0 mg/kg CsA	7.5 mg/kg CsA	30 mg/kg CsA	0 mg/kg CsA	5 mg/kg CsA	30 mg/kg CsA
Crypt depth, μm	80±4	85±5	87±3	96±4	85±4‡	82±3‡
Villus height, μm	394±17	377±16	477±20*†	423±13	403±11	427±17
Villus width at 1/2 height, μm	103±3	90±3	96±6	131±5	133±6	107±5‡#
Villus bottom width, μm	120±4*	118±9	127±7	159±14	142±7	131±6
Villus thickness, μm	591±33	642±25	650±42	399±22	564±25‡	461±35#
Villus surface area, μm^2/villus	600±27	592±27	755±25*†	492±18	630±18‡	523±17#
No. of villi/mm serosal length A	8.39±0.25	8.99±0.76	8.19±0.61	6.69±0.60	7.22±0.41	7.77±0.30
No. of villi/mm serosal length B	1.74±0.10	1.58±0.06	1.61±0.13	2.58±0.15	1.81±0.09‡	2.27±0.15#
No. of villi/mm^2 serosa	14.18±0.43	14.01±1.18	12.61±0.93	16.76±1.51	12.80±0.72‡	16.87±0.65#
Mucosal surface area mm^2/mm^2 serosa	8.53±0.51	8.38±0.82	9.41±0.51	8.20±0.70	8.05±0.45	8.82±0.43

* $p<0.05$ versus 0 mg/kg CsA oral

† $p<0.05$ versus 7.5 mg/kg CsA oral

‡ $p<0.05$ versus 0 mg/kg CsA SQ

# $p<0.05$ versus 5 mg/kg CsA SQ

TABLE IV-5B. ILEAL MORPHOLOGY

	ORAL			SUBCUTANEOUS INJECTION		
Morphological Parameter	0 mg/kg CsA	7.5 mg/kg CsA	30 mg/kg CsA	0 mg/kg CsA	5 mg/kg CsA	30 mg/kg CsA
Crypt depth, μm	76±4	65±2	75±5	64±4	76±4	81±5‡
Villus height, μm	217±14	234±5	197±9†	188±7	164±3‡	211±10‡#
Villus width at 1/2 height, μm	119±9	101±4•	99±3•	73±4	113±4‡	133±6‡#
Villus bottom width, μm	149±15	97±3•	124±6†	88±6	130±5‡	127±6‡
Villus thickness, μm	473±26	453±42	517±41	412±26	486±27	518±31‡
Villus surface area, μm^2/villus	303±19	329±9	283±13	207±9	244±6	349±20‡#
No. of villi/mm serosal length A	71.6±0.64	10.40±0.36•	8.26±0.47†	11.76±0.73	7.80±0.32‡	8.08±0.41‡
No. of villi/mm serosal length B	2.16±0.10	2.09±0.11	2.02±0.12	2.51±0.15	2.12±0.12‡	1.99±0.11‡
No. of villi/mm^2 serosa	15.14±1.35	21.24±0.73•	15.99±0.92†	28.49±1.76	16.05±0.66‡	15.60±0.80‡
Mucosal surface area mm^2/mm^2 serosa	4.45±0.28	7.03±0.39•	4.52±0.30†	5.84±0.33	3.90±0.13‡	5.38±0.27#

• $p<0.05$ versus 0 mg/kg CsA oral

† $p<0.05$ versus 7.5 mg/kg CsA oral

‡ $p<0.05$ versus 0 mg/kg CsA SQ

# $p<0.05$ versus 5 mg/kg CsA SQ

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CHAPTER V

FUNCTIONAL PROPERTIES OF SMALL INTESTINE AFTER SYNGENEIC TRANSPLANTATION IN THE RAT[1]

INTRODUCTION

Recent reports of success with small intestinal transplantation (SIT) and continued improvements in immune suppression techniques have increased interest in the use of small bowel transplantation for treating patients with the short bowel syndrome (SBS) (1,2). An extensive literature has developed regarding the technical and immunological aspects of intestinal transplantation (3-9). However, relatively little is known regarding the functional capacity of the small intestine following orthotopic transplantation. Nutrient absorption from isolated loops (10), in vitro measurements of nutrient uptake (11) and the electrophysiological characteristics of transplanted bowel have been studied (7). Intestinal function, as measured by these parameters was uniformly reduced. However, the majority of these studies were performed using heterotopically-placed intestine not supporting the animal nutritionally or exposed to luminal nutrients. Since defunctioning intestine by itself reduces its functional capacity (12,13), these studies cannot accurately establish the effect of transplantation itself on intestinal function. The limited studies reported using orthotopic models of SIT suggest that transplantation itself reduces nutrient absorption from the diet (14-16), with relatively normal glucose absorption (11). Intestinal function would be an important consideration clinically in determining the length of the graft required. This in turn could affect the immunogenicity of the graft (17) and the possibilities for using living related donors (2).

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[1] A version of this chapter has been submitted for publication to the journal GASTROENTEROLOGY.

MATERIALS AND METHODS

The present study examines the function of orthotopically transplanted intestine in syngeneic rats. We have recently shown that cyclosporine A (CsA) significantly reduces glucose uptake in normal bowel (18,19). A group receiving CsA after syngeneic SIT was included to investigate the effects of CsA on transplanted bowel, not undergoing rejection. Lewis (Lew) rats were used as donors and recipients. Three groups were compared: 1) control animals were normal Lew rats, which received sham injections of the CsA solvent oil; 2) SIT animals underwent orthotopic transplantation of the entire small bowel, and also received sham injections of solvent oil; and 3) SIT plus CsA animals underwent orthotopic SIT, and were then treated with a typical CsA regimen for allogenic SIT in the rat (CsA 15 mg/kg subcutaneously alternate days) (16). We monitored the animal's weight gain and feed intake, and at 2 months performed a balance study to quantify nutrient absorption in vivo. The permeability of the bowel was assessed using four probes of different sizes. The animals were then sacrificed, and the uptake of nutrients in vitro and the electrophysiological characteristics of isolated sections of intestine were determined. This allowed us to correlate in vivo parameters of nutritional well-being with in vitro measurements of the absorptive function of transplanted intestine. Our hypotheses were that transplantation and CsA treatment would independently reduce nutrient uptake by the transplanted bowel.

ANIMALS

Male Lew rats (300-320 g) were obtained from a commercial source (Charles River Canada, St. Constant, PQ), and were housed in individual plexiglass cages, with free access to food (Tekland Premium Lab Diet, Textron Corp., Madison, WI) and water. Feed intake and animal weight were monitored weekly. Weight gain reported is the gain over the final 60 days of the study, following "hook-up" of the transplanted intestine. Day/night cycles were 12 hours, and the temperature was maintained at 20±2°C. Animal care was in accordance with the guidelines of the Canadian Council of Animal Welfare. The experimental protocol used was approved by the Animal Welfare Committee of the University of Alberta.

CYCLOSPORINE

CsA powder was a generous gift of Sandoz Pharmaceuticals (Sandimmune, Sandoz Pharmaceutical Corp., Montreal, PQ). The powder was dissolved in medium chain triglyceride oil (Mead Johnson, Ottawa, ON) at a concentration of 15 mg/mL, and sterilized by microfiltration. Control solution was made up by microfiltering the solvent oil. Animals were injected subcutaneously in the nape of the neck with 15 mg/kg (1.0 mL/kg) of the appropriate solution on alternate days.

TRANSPLANT TECHNIQUE

SIT was performed in two stages. The initial transplant was performed using previously described methods (6,20). In brief, after an overnight fast anaesthesia was induced and maintained using halothane and oxygen via face mask. The entire donor small intestine was isolated on a vascular pedicle that included the portal vein, and the infrarenal aorta. The bowel lumen was flushed in situ with 10 mL of warmed Ringer's Lactate, and the animal was systemically anticoagulated with 150 units of heparin intravenously. The aorta was then ligated above the superior mesenteric artery, flushed with 4 to 6 mL of iced heparinized Ringer's lactate, and the graft was quickly removed and stored in iced Ringer's solution. The recipient's infrarenal cava and aorta were isolated, and the graft was revascularized by anastomosing the portal vein and aorta in turn end to side using 10-0 sutures. The proximal transplanted bowel was then ligated, and the distal ileum was brought out as a stoma. The abdomen was closed, and the animal was immediately allowed free access to food and water.

The second stage of the procedure was done after 2 weeks; a repeat laparotomy was performed, and the native small intestine was resected from the ligament of Treitz to within 1 cm of the ileal-cecal junction. The transplanted bowel was anastomosed in continuity end-to-end with the resected ends of the jejunum and ileum using interrupted sutures of 6-0 silk and an internal stent of macaroni (20). The abdomen was closed, and the animals were allowed free access to water. Food was reintroduced after 24 hours.

NUTRITIONAL STUDIES

Forty-five days following intestinal hook-up, the animals were placed in metabolic cages. After 5 days of preconditioning, they underwent a 3 day balance study, with daily quantitative fecal collections. Total energy, protein and carbohydrate content of feed and feces were determined using standard methods (21,22) and nutrient absorption was calculated directly.

PERMEABILITY STUDIES

Following the balance study, animals underwent assessment of bowel permeability. After an overnight fast, animals were gavaged with a test solution of 10 uCi of ^{51}Cr-EDTA in 2 mL of water. Urine was collected for 6 hours; during this time the animals were allowed free access to water but not to food. After two days, the procedure was repeated using a test solution of mannitol, lactulose, and polyethylene glycol 400 (100 mg of each in a total volume of 2 mL water). Urinary recovery of each marker was then measured using gamma counting for ^{51}Cr, and High Performance Liquid Chromatography for the other markers (23,24)

IN VITRO NUTRIENT UPTAKE AND SHORT CIRCUIT CURRENT

At 60 days post intestinal hook up, animals were sacrificed with a lethal injection of pentobarbital. The bowel was quickly excised, rinsed with iced oxygenated saline and taken for in vitro analysis. The proximal 10 cm of jejunum and the most distal 10 cm of ileum were used for electrophysiological studies, while the remainder of the jejunum and ileum was used for mucosal uptake studies. Representative sections of jejunum and ileum were taken for morphological assessment (25).

The methods used for the determination of the mucosal uptake of nutrients and short circuit current (Isc) have been described in detail previously (26-30), and are reviewed in brief here. Active uptake of D-glucose and passive uptake of fatty acids by the intestinal mucosa was measured using short segments of bowel, opened along the mesenteric border, and carefully washed with a stream of cold saline to remove visible mucus and debris (26,27). The intestine was mounted as a flat sheet in a plastic incubation chamber, with mucosal and serosal surfaces exposed to separate incubation solutions, with apertures in the plates exactly 1.0 cm in diameter. The solutions were mixed at identical stirring rates with circular magnetic bars, with stirring rates precisely adjusted by means of a strobe light. In these studies, the stirring rates were set at 600 rpm to yield low values of the effective resistance of the intestinal unstirred water layer. After preincubation in Krebs-bicarbonate buffer for 15 minutes, the transport chambers were transferred to other beakers containing [^{3}H]-inulin and various probes, including [^{14}C]-labelled D-glucose, L-glucose, cholesterol and fatty acids, in oxygenated Krebs-bicarbonate buffer at 37°C and pH 7.2. The concentrations of D-glucose used were varied from 4-64 mM, and the concentration of L-glucose was 16 mM. The fatty acids used were dodecanoic (12:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3). The long-chain fatty acids (18:0 to 18:3) were prepared in a concentration of 0.1 mM, and were solubilized in 20 mM taurodeoxycholic acid (TDC); cholesterol was prepared in a concentration of 0.05 mM in 20 mM TDC.

After incubation of the intestinal tissue for 6 minutes in one of these test solutions, the experiment was terminated by removing the transport chamber and quickly rinsing the intestinal tissue in cold saline for 5 seconds. The exposed mucosal tissue was then cut out of the chamber with a circular steel punch, and was dried to a constant weight at 55°C. The samples were weighed, saponified with 0.75 N NaOH, scintillation fluid was added and the radioactivity was counted with correction for the variable quenching of the ^3H and ^{14}C isotopes. Samples of jejunum and ileum were done either in duplicate or triplicate for each probe.

ELECTRICAL MEASUREMENTS

Tissues were obtained with the samples for in vitro uptake, and were incubated in iced oxygenated normal Ringer's solution. The intestine was split along the mesenteric border, segments of 2-3 cm were stripped of their serosa and underlying muscle layer, and were mounted in Ussing chambers as previously described (28,29). Normal Ringer's solution with 20 mM fructose was used as the initial incubation solution. The transepithelial electrical potential difference, resistance and Isc were measured over a 15 minute baseline period. Isc changes were then determined with the stepwise addition of D-glucose and theophylline (final concentrations 20 mM and 5 mM respectively). Electrical parameters were monitored for 15 minutes after each addition. Results from jejunum and ileum were recorded in triplicate for each animal, and the results were averaged. Tissues in which resistance varied by greater than 20% were excluded from analysis, and the tissue response to theophylline was used to confirm viability at the completion of the experiment. We have previously demonstrated that measurements of glucose-stimulated Isc correlate with both jejunal and ileal net transmural glucose fluxes (29).

CsA levels were determined at sacrifice of the rat, using a whole blood CsA specific monoclonal antibody assay (Cyclo-trac SP ^{125}I RIA kit, Incstar Corp., Stillwater, MN). Complete blood counts were performed using a Coulter counter (M4-30, Coulter Electronics, Hialeah, FL), and serum electrolyte and creatinine levels were determined using a multistat analyzer (IL-Multistat III, Instrumentation Laboratories, Lexington, MA).

STATISTICS AND ANALYSIS

The kinetic characteristics of D-glucose uptake were determined by plotting uptake versus concentration. Active transport was defined as the observed uptake of D-glucose less the uptake of L-glucose at the same concentration. L-glucose uptake was shown to be a simple linear relation between concentration and uptake, and was calculated accordingly from the known uptake at 16 mM (data not shown). Appropriate corrections were made for the amount of probe present in the adherent mucosal fluid using the ^{3}H inulin marker. Values of the apparent Michaelis constants (Km*) and the maximal transport rate (Vmax) and the associated confidence limits were derived from the resulting curves, from a least squares fit to determine the best line using a statistical package (30). Values were compared using ANOVA, with $p<0.05$ taken as being statistically significant. All results are expressed as mean ± the standard error of the mean (SEM).

RESULTS

GROWTH AND NUTRIENT ABSORPTION

Overall, the animals tolerated the transplant procedures well. Survival of the two operative procedures exceeded 70%. Thereafter feed consumption and growth rate was not affected by SIT or by CsA (Table V-1). The treatments did not appear to affect the animal's general health, and no animals died more than 5 days after an operative procedure. The absorption of fat from the diet was not reduced by transplantation alone, however combined treatment with SIT plus CsA did reduce fat absorption by 7% (Table V-1). Total energy absorption and dry matter digestibility were not affected (Table V-1).

PERMEABILITY

Intestinal permeability was affected by both transplantation and by CsA treatment (Table V-2). After SIT, passive permeability to mannitol and ^{51}Cr-EDTA was increased

(Table V-2). CsA treatment resulted in a further increase in ^{51}Cr-EDTA permeability. The passive permeability to lactulose was not affected by either SIT or CsA treatment and the permeability to PEG-400 was decreased following SIT. The ratios of lactulose/mannitol and PEG-400/mannitol absorption were not changed by these treatments.

NUTRIENT UPTAKE AND ELECTROPHYSIOLOGICAL MEASUREMENTS

In vitro active D-glucose uptake by the enterocyte brush border membrane was affected by both SIT and CsA (Table V-3). In the jejunum, SIT alone increased the Vmax for active D-glucose uptake, while SIT plus CsA negated this increase and returned the Vmax to near-normal levels. The passive uptake of L-glucose was not affected by SIT or CsA treatment. A similar trend was observed in the ileum. Fatty acids and cholesterol uptake were not significantly affected by either SIT or CsA (Table V-4). The exception was dodecanoic acid (12:0), which showed reduced uptake after SIT alone.

Transmural Isc was also affected by SIT (Table V-5). Representative tracings of the Isc response to glucose and theophylline are shown in figure V-1. In the jejunum, SIT reduced the glucose-stimulated change in Isc, without changing the resting Isc or the Isc response to theophylline. Conductance was also decreased by SIT. In the ileum, SIT resulted in a similar reduction in conductance, but did not affect the glucose-induced or theophylline-stimulated changes in Isc. Combined SIT and CsA treatment reduced the ileal glucose-stimulated change in Isc.

Treatments did not affect the animal's CBC, electrolyte or creatinine values (data not shown). CsA levels for the SIT plus CsA group were 507±33 μg/mL. Intestinal morphology was also affected by SIT plus CsA (Table V-6). In these animals, the normal oral-aboral gradient in villus size and mucosal surface area was abolished. The ileal villi were larger, and the mucosal surface area was increased when compared with controls.

DISCUSSION

These results show that while syngeneic intestinal transplants function well enough to allow normal body weight gain, there are significant alterations in the intestinal transport capabilities and increases in the permeability of the small intestine following SIT. Thus, our original hypothesis is partially confirmed: SIT and CsA do affect nutrient transport by the small intestine. However, the general good health of the animals, and the near-normal absorption of dietary nutrients suggest that significant adaptation has occurred. The evidence for this will be discussed after reviewing the implications of the transport data.

The active uptake of D-glucose was clearly increased in the SIT group in the jejunum, with a similar trend in the ileum (Table V-3). However, the changes in Isc induced by glucose were markedly reduced in this same group (Table V-5, Figure V-1). Since the Vmax for D-glucose is directly related to the net activity of the sodium/glucose cotransporter of the enterocyte, while the Isc is related to the net flux of sodium across the intestinal epithelium (31), these results may appear contradictory.

However, a significant proportion of transepithelial sodium flux normally occurs via paracellular (i.e. carrier independent) pathways (32). The alterations in permeability observed after SIT may therefore explain the reductions in glucose-stimulated Isc in these same animals. The increase in intestinal permeability to the smaller probes (mannitol and ^{51}Cr-EDTA) (Table V-2) following SIT suggests that significant back diffusion of sodium and glucose (which are smaller and similar in size respectively to mannitol) (33,34) from the paracellular spaces to the intestinal lumen may occur after SIT. Thus, glucose and sodium that are cotransported across the enterocyte into the intracellular space could diffuse back into the intestinal lumen, reducing the net flux of sodium (as suggested from the measurement of the glucose-stimulated Isc). Alternatively, the observed reduction in glucose-stimulated Isc may have resulted from a decrease in conductivity of the subepithelial layers of the intestine. Such changes have been described following adaptation after massive resection (35). Direct measurement of the transmural flux of glucose and electrolytes is required to clarify this matter.

An alternative explanation for the observed alterations in Isc may involve changes in the activity of the chloride-secreting pump of the intestinal crypt cells (36). Previous investigators have shown that SIT results in increased basal chloride secretion by this pathway, and reduced uptake of luminal sodium and chloride (8). This is thought to be the result of intestinal denervation, specifically the loss of the normal sympathetic inhibition of the enteric nervous system (36). It is probable that this loss of sympathetic input persists after SIT (36,37), although reinervation may occur (38). It is difficult to quantify the activity of the enteric nervous system directly, however transplanted intestine has been shown to have normal patterns of myoelectrical activity and normal intrinsic reflexes, but abnormal integration with the native bowel activity and absent extrinsic inhibition (39,40). Chronic changes in the activity of enteric ganglia could change the transport properties of the intestine by reducing the tonic inhibition of crypt chloride secretion, and decreasing villus uptake of sodium and chloride. Altering the flux of sodium in this way could then secondarily affect many other uptake pathways in the small intestine (31).

Changes in the activity of the enteric nervous system may also explain the observed decrease in conductance following SIT (Table V-4). Cyclic-AMP is a ubiquitous second messenger in the gastrointestinal tract; with an increase in intermucosal neural activity after SIT, cyclic-AMP levels could reasonably be expected to increase. Acute increases in cyclic-AMP levels have been shown to decrease conductance in normal rabbit ileum (41). Thus, alterations in the activity of the enteric nervous system may link changes in intestinal transport and electrical characteristics following SIT. Further studies are required to clarify these relationships.

The increase in absorption of mannitol and ^{51}Cr-EDTA following SIT demonstrates an alteration in mucosal permeability (34). Permeability to a marker is thought to reflect the "accessibility" of ~~the~~ probe to physiological and pathological breaks in the intestinal epithelium, and to the potential for direct uptake into the brush border membrane. The physiological breaks in the epithelium are transmembrane pores and gaps in the "tight junctions" (42,43). Smaller molecules such as mannitol can traverse both pores and gaps in the tight junctions, while larger molecules such as ^{51}Cr-EDTA and lactulose permeate via gaps in the tight junctions. PEG-400 is thought to penetrate the intestinal epithelium via tight junctions and possibly by direct dissolution in the lipid membrane (43). The pattern of mucosal uptake of mannitol and ^{51}Cr-EDTA following SIT suggests effects at the tight junctions. However, these must be specific in size, because the permeability to lactulose and PEG-400 was not increased. The decrease in permeability to PEG-400 following SIT is difficult to explain on the basis of changes in the tight junctions alone. Furthermore, the in vitro uptake of lipids was not affected by transplantation (Table V-4) so that alterations in lipophilic uptake of PEG-400 are not likely to alter its absorption. Motility changes have been implicated as a cause of alterations in the absorption of markers (34), and since changes in the myoelectrical activity of the bowel have been well described following SIT (39,40), this may have affected permeability measurements in the present study. It is unlikely that mucosal permeability was affected by the transplantation operation itself, since the transport and permeability studies were done after perioperative hypoxic damage should have resolved (13). It is more probable that the alteration in mucosal permeability was caused by transplant induced changes in intracellular tight junctions (32). Previous studies have shown that the morphology and ultrastructure of syngeneically transplanted small intestine appeared normal (7,44,45), however these studies were limited to the first 14 days following transplantation. A number of the factors which have been discussed in relation to transport function, including enterocyte membrane composition, cyclic-AMP levels, and enteric neural activity can affect permeability by altering the intracellular tight junctions (32). More direct studies of this matter are required, especially since changes in permeability have been used as a marker of rejection in allogeneic intestinal transplants (1,23).

The reductions in glucose-induced changes in Isc which occurred suggest that transmural uptake of glucose should be reduced following SIT (29,30). As noted, the near normal absorption of dietary total energy, and normal weight gain following SIT suggest that the animals are able to compensate for this. The colon, which is preserved in this model, may be a factor, in that it can compensate for small intestinal losses of water, electrolyte, and short-chain fatty acids, however it cannot adapt to absorb glucose (46). The normal absorption of dietary total energy suggests that the small intestine has undergone adaptation following SIT. The mechanism for this may be an increase in the number of sodium-glucose cotransport carriers per enterocyte; this would explain the increase in the Vmax for active glucose uptake observed (Table V-3) (13,47). Adaptation may have also stimulated the increase in villus size and mucosal surface area in the SIT plus CsA group (13,46). This group of animals had persistent malabsorption of fat (Table V-1), and did not show an increase in the Vmax for glucose uptake (Table V-3), but yet did not malabsorb energy. This suggests that the denervated intestine responded to the decrease in energy availability induced by transplantation and CsA treatment by stimulating an increase in absorptive surface area. This pattern of adaptation is similar to that described with other signals for adaptation, such as intestinal resection (27), diabetes (29), and irradiation (13). While the mechanisms underlying these changes require clarification, it is significant that adaptation can occur without changes in feed intake, humeral factors, or extrinsic innervation, all of which have been suggested as putative signals for the adaptive response (13,47).

The near normal absorption of dietary fat following SIT noted in these studies is somewhat surprising, considering the findings of previous authors (14-16). The lack of effect in the present study may be due to the low fat diet used (4% of total calories), and the timing of the studies. Previous investigators have shown that fat malabsorption decreases as the lymphatic connections of the bowel regenerate; in the rat this occurs within the first month of transplantation (14,48). The minimal effects of transplantation on fatty acid and cholesterol uptake in vitro would further suggest that transplantation does not have a major effect on enterocyte membrane composition, or the unstirred water layer.

CsA caused further significant reductions in function in syngeneically transplanted small intestine. It abolished the increase in jejunal glucose uptake following SIT (Table V-3). It also increased intestinal permeability to ^{51}Cr-EDTA, and reduced dietary fat absorption. These changes are similar to those we have observed in normal bowel (13,14), and suggest that transplantation does not alter the sensitivity of the bowel to the inhibitory effects of CsA on nutrient transport. As well, CsA has been shown to impair the regrowth of lymphatus after SIT (48). Further study is required to establish if these effects occur in other models, especially those whose allogeneic SIT mandates the long-term use of CsA.

Syngeneic transplantation of the small intestine results in a significant change in the functional capacity of the small bowel. These studies also demonstrate the utility of using syngeneic SIT as a model for studying the interactions between the enteric nervous system and epithelial function. The functional changes observed require consideration in attempts at clinical SIT; specifically with regard to the amount of intestine transplanted and the use of living related donors. Nevertheless the general good health of the transplanted animals demonstrates that SIT is a promising modality for clinical application.

TABLE V-1: Animal characteristics following syngeneic small intestinal transplantation.

	Controls	Transplant	Transplant plus CsA
n	8	9	10
Initial weight (g)	318±8.9	313±10.7	295±8.5
Weight gain (g/60 days)	105±4.4	113±8.7	108±8
Feed intake (g/day)	21.2±1.2	27.3±1.0	20.4±1.1
NUTRIENT ABSORPTION (% absorbed from diet)			
Total energy	84±0.4	84±0.5	83±0.2
Fat	79±0.7	77.7±0.6	74.9±1.0*
Dry matter	80±0.6	79.6±0.6	79.8±0.6

* $p<0.05$ versus controls

TABLE V-2: Intestinal permeability following syngeneic small intestinal transplantation‡.

Marker	Controls	Transplant	Transplant plus CsA
n	8	9	10
^{51}Cr-EDTA	2.1±0.15	2.7±0.1*	4.8±0.6*†
Mannitol	2.3±0.2	4.9±8*	5.3±0.4*
Lactulose	1.2±0.5	0.9±0.3	0.9±0.2
PEG 400	22±2.5	11.8±1.8*	13.4±0.9*

* p<0.05 versus controls

† p,0.05 versus transplanted animals

‡ % recovery of orally administered marker in urine

TABLE V-3: Intestinal transplantation effects on the kinetics of glucose uptake.

	Controls	Transplant	Transplant plus CsA
n	8	9	10
JEJUNUM			
Vmax	22.0±3.0	43.9±4.7*	22.3±2.7†
Km*	1.6±1.2	1.6±1.1	3.5±1.5
ILEUM			
Vmax	17±3.3	25.4±3.5	18.1±2.1
Km*	4.1±2.9	2.6±1.5	6.9±0.8

Units: Vmax nmoles·cm^{-2} mucosa·min^{-1}, Km* = mM

* $p<0.05$ versus controls

† $p<0.05$ versus transplant alone

TABLE V-4: In vitro lipid uptake following syngeneic small intestinal transplantation.†

LIPID	Control Lew rats	Transplant Lew-Lew	Transplant and CsA Lew-Lew
JEJUNUM			
12:0	11.0±3.3	4.4±1.5	6.5±1.2
18:0	0.8±0.1	0.7±0.1	0.5±0.1
18:1	0.9±0.2	0.9±0.3	0.7±0.1
18:2	0.6±0.1	1.0±0.3	0.6±0.1
18:3	0.8±0.2	0.6±0.1	0.5±0.1
Cholesterol	1.1±0.2	0.9±0.1	0.9±0.1
ILEUM			
12:0	22.0±4.1	6.3±1.7*	10.9±3.0
18:0	0.6±0.1	0.9±0.1	0.5±0.1
18:1	0.4±0.2	1.0±0.2	0.5±0.1
18:2	0.7±0.3	0.8±0.1	0.6±0.1
18:3	0.8±0.2	0.6±0.2	0.5±0.1
Cholesterol	1.0±0.2	1.0±0.2	0.9±0.1

† units = nmol/100 mg tissue/min

* $p<0.05$ transplant versus controls

TABLE V-5: Intestinal short circuit current following small intestinal transplantation.

	Controls	Transplant	Transplant plus CsA
n	8	9	10
JEJUNUM			
Glucose-induced Isc (20 mM)	63.4±5.1	32.7±3.2*	29.0±5.0*
Theophyline-induced Isc (5 mM)	37.9±3.3	28.2±4.9	26.9±2.6
Conductance (cm^2/ohm)	21.9±2.5	14.9±0.3*	13.2±0.3*
Resting Isc	34.3±3.8	29.0±3.2	24.9±2.8
ILEUM			
Glucose-induced Isc (20 mM)	42.0±3.0	44.0±1.3	22.1±3.9*
Theophyline-induced Isc (5 mM)	41.6±3.0	33.3±2.1	34.4±1.3
Conductance (cm^2/ohm)	20.5±2.0	15.1±0.4*	14.1±0.7*
Resting Isc	30.9±2.2	24.2±1.8*	22.0±3.0

Short circuit current (Isc) values expressed as •amp/cm^2 mucosa.

Glucose/theophline-Induced Isc = change in Isc after addition of substrate

* $p<0.05$ versus controls

TABLE V-6: Intestinal morphology following syngeneic small intestinal transplantation.

TABLE V-6A. JEJUNAL MORPHOLOGY

MORPHOLOGICAL PARAMETER	Control Lew	Transplant Lew-Lew	Transplant + CsA Lew-Lew
Crypt depth, μm	96±4	85±6	65±5*†
Villus height, μm	423±13	448±38	459±20
Villus width, at 1/2 height, μm	131±5	100±1†	97±3†
Villus bottom width, μm	159±14	134±6	122±4†
Villus thickness, μm	399±22	438±29	613±47*†
Villus surface area μm^2/villus	492±18	510±50	696±31*†
No. of villus/mm serosal length A	6.69±0.60	7.58±0.34	8.32±0.28†
No. of villus/mm serosal length B	2.58±0.15	2.38±0.16	1.72±0.13*†
No. of villus/mm^2 serosa	16.76±1.51	17.30±0.77	13.58±0.46
Mucosal surface area mm^2/mm^2 serosa	8.20±0.70	8.74±0.66	9.49±0.63

* $p<0.05$, transplant plus CsA versus transplantation

† $p<0.05$, transplant versus controls

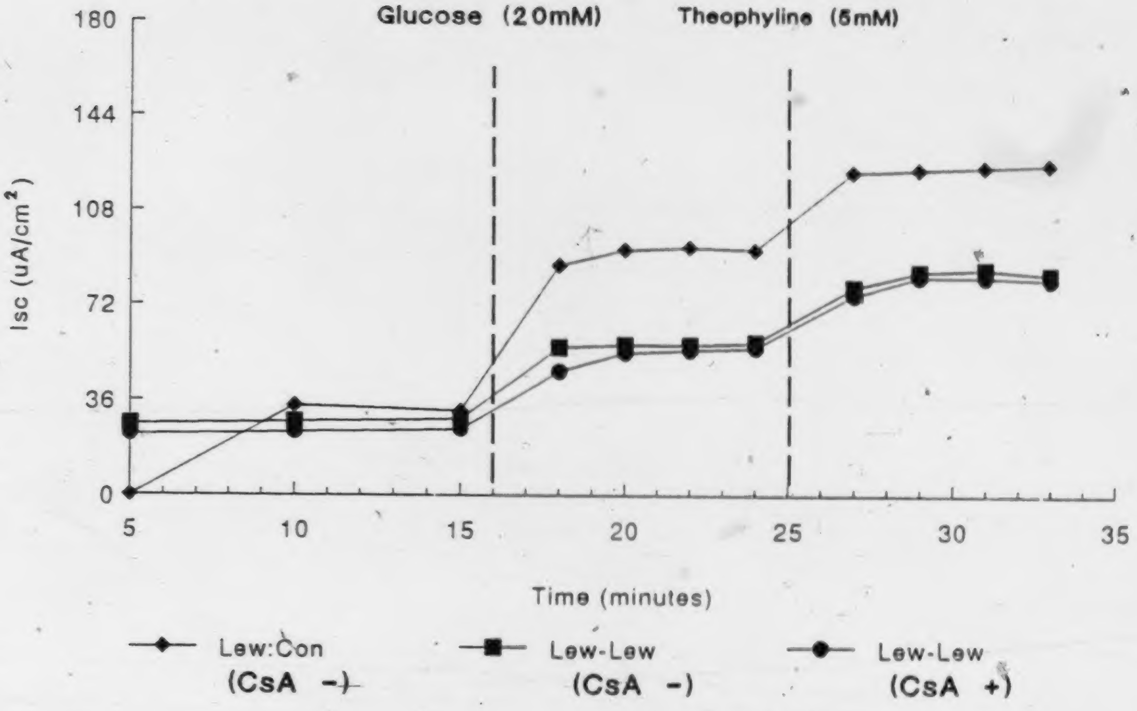
TABLE V-6B. ILEAL MORPHOLOGY

MORPHOLOGICAL PARAMETER	Control Lew	Transplant Lew-Lew	Transplant + CsA Lew-Lew
Crypt depth, μm	64±4	61±4	71±5
Villus height, μm	188±7	174±4	312±13*†
Villus width, at 1/2 height, μm	73±4	94±5†	86±2†
Villus bottom width, μm	88±6	103±6	80±4*
Villus thickness, μm	412±26	423±19	468±27
Villus surface area μm^2/villus	207±9	216±8	389±16*†
No. of villus/mm serosal length A	11.76±0.73	10.01±0.64	12.9±0.72*
No. of villus/mm serosal length B	2.51±0.15	2.41±0.11	2.21±0.14
No. of villus/mm^2 serosa	28.49±1.76	23.64±1.50	27.57±1.55
Mucosal surface area mm^2/mm^2 serosa	5.84±0.33	5.07±0.29	10.74±0.7*†

* $p<0.05$, transplant and CsA versus transplantation

† $p<0.05$, transplant versus controls

FIGURE V-1: Intestinal short circuit current: changes with glucose and theophylline following syngeneic small intestinal transplantation.



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CHAPTER VI

INTESTINAL FUNCTION FOLLOWING ALLOGENEIC SMALL INTESTINAL TRANSPLANTATION IN THE RAT[1]

INTRODUCTION

Continued improvements in immune suppression techniques have resulted in recent reports of clinical success with small intestinal transplantation (SIT) in man (1,2). In all likelihood, the future will bring further refinements in immunosuppressive drugs and treatments that will allow greater application of SIT for patients with inadequate gastrointestinal absorptive capacity (3). Previous studies have shown that following orthotopic intestinal transplantation there is prolonged malabsorption of dietary nutrients, especially fat (4,5), and the uptake of glucose, glycine, and electrolytes from isolated loops of bowel is reduced (6). Other authors have shown that in vitro glucose uptake (7) and the electrophysiological characteristics of transplanted bowel (8,9) are relatively normal in short-term studies of non-rejecting bowel. However, the majority of these studies were performed on isolated Thiry-villa fistulas, which is not supporting an animal nutritionally. Since absorptive function following intestinal transplantation is the most clinically important physiological determinant of graft function, we undertook the present studies of intestinal function after orthotopic SIT between fully allogeneic and syngeneic strains of rats. Reduced nutrient absorption following SIT would affect such parameters as the length of bowel required and the bioavailability of orally administered medications (10). Increasing the length of the grafted intestine could affect the immunogenicity of the graft (11), and the possibilities for using living related donors (2).

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[1] A version of this chapter has been submitted for publication to the journal TRANSPLANTATION.

The effects of transplantation itself, without rejection, were determined by including two groups of rats undergoing syngeneic SIT. We have previously demonstrated that intestine from normal Brown Norway (BN) rats differs from Lewis (Lew) intestine in glucose transport characteristics (Sigalet DL, unpublished observations). Accordingly, the transport characteristics of the allogeneically transplanted BN bowel (BN→Lew) were compared to both normal BN small intestine and (BN→BN) syngeneically transplanted small intestine. However, normal BN rats grow at a slower rate than normal Lew rats, and so the growth characteristics of the Lew animals receiving the allogeneic transplants were compared to (Lew→Lew) SIT animals. Because we have recently shown that cyclosporine A (CsA) treatment itself affects nutrient uptake by the bowel (12,13), all animals received CsA throughout the study protocol. Four groups were compared: group 1) control (BN:Con) animals were normal BN rats (n=8); group 2) BN syngeneic SIT (BN→BN) was performed between BN donors and BN recipients (n=9); group 3) Lew syngeneic SIT (Lew→Lew) was performed between Lew donors and recipients (n=10); group 4) allogeneic SIT (BN→Lew) was performed between BN donors and Lew recipients (n=10).

Intestinal function was monitored using a variety of parameters, including general animal well-being, weight gain, and feed intake. At 50 days following intestinal reanastomosis, dietary nutrient absorption was quantified using an in vivo balance study. The permeability of the bowel was assessed using four probes of different sizes. The animals were then sacrificed, and the uptake of nutrients in vitro and the electrophysiological characteristics of isolated sections of intestine were determined. This allowed us to correlate in vivo parameters of nutritional well being with in vitro measurements of the absorptive function of transplanted intestine. Our hypothesis was that transplantation itself and the immune events associated with allogeneic transplantation would independently reduce nutrient uptake in the fully allogeneic transplanted bowel.

MATERIALS AND METHODS

ANIMALS

Male BN and Lew rats (280-310 g) were obtained from a commercial source (Charles River Canada, St. Constant, PQ), and were housed in individual plexiglass cages, with free access to food (Tekland Premium Lab Diet, Textron Corp., Madison, WI) and water. Feed intake and animal weight were monitored weekly. Weight gain is described relative to the initial pretransplant weight for transplanted animals, and the weight at the start of the 60 day test period for the BN:controls. The guidelines of the Canadian Council of Animal Welfare were followed for animal care. The experimental protocol used was approved by the Animal Welfare Committee of the University of Alberta.

CYCLOSPORINE

CsA in powder form was a generous gift of Sandoz Pharmaceuticals, (Sandimmune, Sandoz Pharmaceutical Corp., Montreal, PQ). The powder was dissolved in medium chain triglyceride oil (Mead Johnson, Ottawa, ON) at a concentration of 15 mg/mL, and sterilized by microfiltration. Animals were injected subcutaneously in the nape of the neck with 15 mg/kg body weight (1.0 mL/kg) of the CsA solution just prior to transplantation, then daily for 6 days, and on alternate days thereafter (4).

CsA levels were determined at the time of sacrifice of the rat, using a whole blood CsA specific monoclonal antibody assay (Cyclo-trac SP ^{125}I RIA kit, Incstar Corp., Stillwater, MN).

Complete blood counts were performed using a Coulter counter (M4-30, Coulter Electronics, Hialeah, FL), and serum electrolyte and creatinine levels were determined using a multistat analyzer (IL-Multistat III, Instrumentation Laboratories, Lexington, MA).

TRANSPLANTATION

A two-stage technique of SIT was used (14). The initial transplant was performed using previously described methods (14,15). In brief, after an overnight fast, anesthesia was induced and maintained using halothane and oxygen via face mask. The entire donor small intestine was isolated on a vascular pedicle that included the portal vein and the infrarenal aorta. The bowel lumen was flushed in situ with 10 mL of warmed (37°C) Ringer's lactate, and the animal was systemically anticoagulated with 150 Units of heparin given intravenously. The aorta was then ligated above the superior mesenteric artery, flushed with 4-6 mL of iced heparinized Ringer's lactate, and the graft quickly removed and stored in iced Ringer's solution. The recipient's infrarenal cava and aorta were isolated, and the graft was revascularized by anastomosing the portal vein and aorta in turn end-to-side using 10-0 sutures. The proximal transplanted bowel was then ligated, and the distal ileum was brought out as a stoma. The abdomen was closed, and the animal was allowed free access to food and water immediately.

The second stage of the procedure was done after 12-16 days; a repeat laparotomy was performed, and the native small intestine was resected from the ligament of Treitz to within 1 cm of the ileal-cecal junction. The transplanted bowel was anastomosed in continuity end-to-end with the resected ends of the jejunum and ileum using interrupted sutures of 6-0 silk and an internal stent of macaroni (14). The abdomen was closed, and the animals were allowed free access to water immediately. Food was reintroduced after 24 hours. Animals dying within 5 days of an operation were considered technical failures and were replaced.

NUTRITIONAL STUDIES

Forty-five days following intestinal reanastomosis, the animals were placed in metabolic cages. After 5 days of preconditioning, they underwent a 3 day in vivo balance study, which entailed daily quantitative fecal collections, and exact determination of the feed intake. Total energy, protein, and carbohydrate content of feed and feces were determined using standard methods (16,17) and nutrient absorption was calculated directly.

PERMEABILITY STUDIES

On day 55, following the balance study, animals underwent assessment of bowel permeability, as measured by the urinary excretion of a variety of orally administered probes. After an overnight fast, animals were gavaged with a test solution of 10 uCi of ^{51}Cr-EDTA in 2 mL of water. Urine was collected for 6 hours; during this time the animals were allowed free access to water but not to food. After 2 days, the procedure was repeated using a test solution of mannitol, lactulose, and polyethylene glycol 400 (100 mg of each in a total volume of 2 mL water). Urinary recovery of each marker was then measured using gamma counting for ^{51}Cr-EDTA, and High Performance Liquid Chromatography for the other markers (18,19)

IN VITRO NUTRIENT UPTAKE

Sixty days following intestinal reanastomosis, the animals were sacrificed with a lethal injection of pentobarbitol. The transplanted intestine was quickly excised, rinsed with iced oxygenated saline, and was used for in vitro studies: the proximal 10 cm of jejunum and the most distal 10 cm of ileum were used for electrophysiological studies, while the remainder of the jejunum and ileum was used for mucosal uptake studies. Representative sections of jejunum and ileum were taken for morphological assessment (20).

The methods used for the determination of the mucosal uptake of nutrients and short circuit current (Isc) have been described in detail previously (21-24), and are reviewed in brief here. Active in vitro uptake of D-glucose and passive uptake of fatty acids by the intestinal mucosa was measured using short segments of bowel, opened along the mesenteric border, and carefully washed with a stream of cold saline to remove visible mucus and debris (21,22). The intestine was mounted as a flat sheet in a plastic incubation chamber, with mucosal and serosal surfaces exposed to separate incubation solutions, with apertures in the plates exactly 1.0 cm in diameter. The solutions were mixed at identical stirring rates with circular magnetic bars, with stirring rates precisely adjusted by means of a strobe light. For these studies, the stirring rates were set at 600 rpm, which yields low values for the effective resistance of the intestinal unstirred water layer. After preincubation in Krebs-bicarbonate buffer for 15 minutes, the transport chambers were transferred to other beakers containing [^{3}H]-inulin and various probes, including [^{14}C]-labelled D-glucose, L-glucose, cholesterol and fatty acids, in oxygenated Krebs-bicarbonate buffer at 37°C and pH 7.2. The concentrations of D-glucose used were varied from 4-64 mM, and the concentration of L-glucose was 16 mM. The fatty acids used were dodecanoic (12:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3). The dodecanoic acid was prepared at a concentration of 0.2 mM. The long-chain fatty acids (18:0 to 18:3) were prepared in a concentration of 0.1 mM, and were solubilized in 20 mM taurodeoxycholic acid (TDC); cholesterol was prepared in a concentration of 0.05 mM in 20 mM TDC.

After incubating the intestinal tissue for 6 minutes in one of these test solutions, the experiment was terminated by removing the transport chamber and quickly rinsing the intestinal tissue in cold saline for 5 seconds. The exposed mucosal tissue was then cut out of the chamber with a circular steel punch, and was dried to a constant weight at 55°C. The samples were weighed, saponified with 0.75 N NaOH, scintillation fluid was added, and the radioactivity was counted with correction for the variable quenching of the ^{3}H and ^{14}C isotopes. Samples of jejunum and ileum were done either in duplicate or triplicate for each probe. Active uptake of glucose is expressed as nmoles·cm^2 mucosa^{-1}·min^{-1} to facilitate comparisons with the electrophysiologic data, while passive uptake of fatty acids is expressed as nmoles·100 mg mucosa^{-1}·min^{-1}.

ELECTRICAL MEASUREMENTS

Tissues were obtained with the samples for in vitro uptake, and were incubated in iced oxygenated normal Ringer's solution. The intestine was split along the mesenteric border, segments of 2-3 cm were stripped of their serosa and underlying muscle layer, and were mounted in Ussing chambers as previously described (23,24). Normal Ringer's solution with 20 mM fructose was used as the initial incubation solution. The transepithelial electrical potential difference, resistance and Isc were measured over a 15 minute baseline period. Isc changes were then determined with the stepwise addition of D-glucose and theophylline (final concentrations 20 mM and 5 mM respectively). Electrical parameters were monitored for 15 minutes after each addition. Results from jejunum and ileum were recorded in triplicate for each animal, and the results were averaged. Tissues in which resistance varied by greater than 20% were excluded from analysis, and the tissue response to theophylline was used to confirm viability at the completion of the experiment. We have previously demonstrated that measurements of glucose induced Isc correlate with both jejunal and ileal net transmural fluxes of glucose (24).

STATISTICS AND ANALYSIS

The kinetic characteristics of D-glucose uptake were determined by plotting uptake versus concentration. Active transport was defined as the observed uptake of D-glucose less the uptake of L-glucose at the same concentration. L-glucose uptake was shown to be a simple linear relation between concentration and uptake, and was calculated accordingly from the known uptake at 16 mM (data not shown). Appropriate corrections were made for the amount of probe present in the adherent mucosal fluid using the ^{3}H inulin marker. Values of the apparent Michaelis constants (Km*), the maximal transport rate (Vmax), and the associated confidence limits were derived from the resulting curves, from a least squares fit to determine the best line using a statistical package (25). Values were compared using ANOVA, with $p<0.05$ taken as being statistically significant. All results are expressed as mean ± the standard error of the mean (SEM).

RESULTS

GENERAL WELL BEING, GROWTH, AND NUTRIENT ABSORPTION

Survival of the two operative procedures exceeded 75%. There were two late deaths in the syngeneic groups: one animal died after volvulus of the transplanted bowel, while the second animal died of pneumonia after aspirating the permeability test solution and died. There were three late deaths in the allogeneic transplant groups: two animals developed pneumonia more than 30 days after intestinal reanastomosis, and one other animal died of graft arterial thrombosis 2 weeks after intestinal reanastomosis. A fourth animal was found at sacrifice to have a stricture at the distal intestinal anastomosis, and was excluded from data analysis. There was no evidence of graft rejection at necropsy in these four animals. This left data from eight BN:controls, seven (BN→BN) syngeneic SIT, ten (Lew→Lew) syngeneic SIT, and six (BN→Lew) allogeneic SIT animals for consideration. The BN→Lew animals went through a distinctive phase of graft-versus-host-disease (GVHD) during the eighth to fourteenth posttransplant days, similar to that described by previous authors (15,26). This was characterized by red ears and paws, hunched posture, and decreased feed intake, but minimal diarrhea. In all cases, this had resolved with the continued CsA treatment by day 16 posttransplantation, prior to intestinal reanastomsosis.

Initial weight gain was reduced in the transplanted animals in each group (Table VI-1, Figure VI-1). Previous workers have shown that weight gain is near normal following syngeneic SIT in the Lew rat (4); this has been confirmed by our own observations

(Chapter V). However, in the present studies, BN→BN syngeneic SIT resulted in a significant reduction in total weight gain, demonstrating a strain specific effect of SIT. Furthermore, the total weight gain following allogeneic SIT (BN→Lew) was significantly less than after syngeneic SIT (Lew→Lew), demonstrating that Lew recipients do not fare as well after receiving an allogeneic graft as compared to a syngeneic transplant. It is not clear from these studies what caused this reduction in weight gain following allogeneic SIT, however the reduced rate of weight gain improves with time (Figure VI-1). Fourteen days after reestablishing gastrointestinal continuity with the transplanted intestine, the rate of weight gain of the BN→Lew group approached that of the Lew→Lew SIT group (Table VI-1).

Nutrient absorption was examined on days 50-53 following intestinal reanastomosis. Fat absorption was significantly reduced in all the transplanted animals (Table VI-1), with a further reduction in the allogeneic SIT group, when compared to the syngeneic BN→BN animals. Energy absorption from the diet was reduced after transplantation; this difference was significant in the syngeneic BN→BN group (Table VI-1).

INTESTINAL PERMEABILITY

All of the transplanted animals had a similar increase in the permeability to mannitol and PEG-400 while the allogeneic SIT group had a further increase in permeability to mannitol, lactulose PEG-400, and ^{51}Cr-EDTA (Table VI-2). The SIT associated increase in the permeability to mannitol decreased the ratio of the permeabilities of lactulose/mannitol and ^{51}Cr-EDTA/mannitol in the syngeneic SIT groups, when compared with the BN:controls. The increase in permeability to the larger probes (^{51}Cr-EDTA and lactulose) increased, but did not normalize these ratios in the allogeneic group.

IN VITRO NUTRIENT UPTAKE

Both allogeneic and syngeneic SIT affected in vitro active glucose uptake (Table VI-3). The Vmax for glucose in the jejunum and ileum was increased in both the BN→BN and

BN→Lew groups, when compared to the BN controls. The passive uptake of L-glucose was not affected by transplantation (data not shown). This data is reported as uptake per unit surface area of the bowel, which allows for direct comparison to the electrophysiological data which follows. However, the results were similar when uptake was reported per unit weight of intestinal mucosa (data not shown).

Fatty acid uptake was also affected by SIT (Table VI-4). Dodecanoic acid (12:0) uptake was reduced in the allogeneic SIT (BN→Lew) group. The long-chain fatty acids (18:0, 18:1, 18:2, 18:3) showed a consistent pattern of increased uptake in the BN→Lew animals, and a further increase in the syngeneic BN→BN group, as compared to the BN:controls. A similar pattern was observed in the ileum. The most significant change in uptake was seen with cholesterol (BN→BN uptake 5.2±1.2 versus BN:controls 2.8±0.6, nmoles/100 mg tissue/min, $p<0.05$) The remainder of differences did not reach significance (Table VI-4).

ELECTROPHYSIOLOGIC PARAMETERS

The major electrophysiological effect of SIT was to reduce the glucose-stimulated change in Isc (Table VI-5, Figure VI-2). Both syngeneic (BN→BN) and allogeneic (BN→Lew) transplants of BN intestine had similar reductions in jejunal and ileal glucose-stimulated Isc values, without changes in the resting, or theophylline-stimulated Isc. Jejunal conductance was reduced in all the transplanted animals, while ileal conductance was not. As noted in the discussion of the experimental design, the small intestine of normal BN and Lew animals is different; the transport characteristics of BN intestine after BN→Lew transplantation were compared to Lew→Lew transplanted intestine to determine if BN bowel retained these differences in the Lew recipient. The in vitro active uptake of glucose in the BN→Lew group was significantly greater than in the Lew→Lew animals. Lew→Lew transplanted small intestine showed a trend towards increased in vitro active glucose uptake compared with normal Lew animals treated with CsA (Table V-3) which is similar to the effect of transplantation on BN intestine after BN→BN SIT. The glucose-induced Isc changes were reduced to a similar extent in all transplanted groups. The pattern of changes suggests that BN bowel retains its intrinsic transport characteristics after transplantation into Lew animals. Thus, transplantation itself induced an increase in the mucosal Vmax for glucose uptake, and reduced the glucose-induced Isc. Allogeneic SIT did not appear to affect these parameters further, except for the apparent increase in the ileal Km* for glucose.

MORPHOLOGY

There was no change in the jejunal morphology of the BN bowel following transplantation (Table VI-6). In the ileum, there was a modest increase in villus size and mucosal surface area following transplantation. There was no evidence of rejection or GVHD in the BN→Lew group at sacrifice.

The hematological, electrolyte and creatinine values were not significantly different in any of the treated groups (data not shown). CsA levels were also similar in each group and averaged 630±33 ug/mL.

DISCUSSION

Previous studies have suggested that the transport properties (8,9) and permeability of non-rejecting small intestine (18) are normal following SIT. In contrast, this study demonstrates alterations in these parameters of intestinal function following SIT. However, these alterations have a surprisingly modest impact on the overall well-being of the animal. BN→BN and BN→Lew SIT was associated with a reduction in body weight gain over the study period (Table VI-1), while Lew→Lew SIT animals grew at a normal rate for Lew rats (Table V-1).

Evaluating the factors which may have affected the weight gain in these animals, there was no difference in the feed intake (Table VI-1). The basal metabolic rate of the animals was not quantified in these studies, however within strains, growth rates are very predictable provided nutrient availability is equivalent (28). In the assessment of dietary nutrient absorption performed at day 50, fat absorption was decreased in all of the groups after SIT, and absorption of total energy (primarily carbohydrate in the diet used) was decreased in the BN→BN syngeneic SIT group. This parallels the experience reported by previous authors (3,4,29).

The lag in weight gain noted in the allogenic BN→Lew transplanted animals is an interesting phenomenon; after this 14 day period, the rate of growth was normal in these animals (Figure VI-1, Table VI-1). No direct measurements were done during this period to quantify nutrient handling, except measurement of feed intake, which was not affected. However this lag period coincided with the time period when the animals were recovering from GVHD. Previous authors have noted that with short-term CsA treatment, GVHD occurs 4 to 6 weeks following SIT (30). With long-term CsA, no such delayed GVHD was observed (4). More direct studies of the impact of GVHD on nutrient absorption are necessary. However the implication of these observations is that following allogeneic SIT a decrease in nutrient absorption (or increase in metabolic rate) occurs which may correspond to the repair of GVHD effects. Following this lag period, bowel function is adequate to support normal rates of growth.

The relatively normal absorption of dietary total energy by all groups following SIT is interesting in light of the changes in Vmax and Isc values noted after SIT. The Vmax for glucose uptake was increased in all groups following SIT (Table VI-3). Because of the cotransport of sodium and glucose, it was anticipated that the glucose-stimulated Isc would be greater in the SIT groups when compared to the control animals (27,31), but the converse was true (Table VI-5). The resting Isc was not affected by SIT, suggesting that alterations in the sodium gradient across the basolateral membrane was not the explanation for the lack of expected increase in the glucose-stimulated Isc. It is possible that SIT affected the glucose-induced activity of the Na^+/K^+ ATPase of the BLM, but this was not examined in the present study. Alternatively, the reduced glucose-stimulated Isc may have been due to an increase in the "back flux" of sodium, from the paracellular space to the intestinal lumen; although the net flux of sodium was not quantified in this study, we did note an increase in intestinal permeability (Table VI-2) which supports this hypothesis. An alternative or additive cause of the reduction in glucose-stimulated Isc may be a transplant induced increase in the resistance of the subepithelial tissues (primarily the basement membrane). Adaptation following bowel resection has been shown to stimulate such an increase in resistance (32), which may mask increases in glucose-stimulated Isc. The basement membrane is a dynamic structure, which is affected by a variety of stimuli for intestinal adaptation (33). The net decrease in conductance (inverse of resistance) noted in the jejunum (Table VI-5) following SIT may reflect a similar adaptive response.

Our findings of a consistent reduction in glucose-stimulated Isc following SIT contrasts with previous studies in which glucose-stimulated Isc values were normal following SIT (8,9). In these previous reports, Isc evaluations were done at 3, 6 and 9 days posttransplantation, using isolated Thiry-villa fistulas and preparations of intestine which were not stripped of their serosa. These conditions would tend to minimize alterations in glucose-stimulated Isc (23,34).

Permeability of the intestine, as measured by the probes used in this study, is thought to reflect the ability of the marker to passively diffuse through the intestinal epithelium by way of tight junctions, aqueous pores, and possibly the lipophilic properties of the brush border membrane (35-38). Mannitol may permeate both pores and tight junctions, while lactulose, ^{51}Cr-EDTA, and probably PEG-400 (37) diffuse exclusively via the tight junctions. It has been suggested that the best predictor of permeability is molecular size and polarity

(38). On this basis, one might have predicted that the intestinal permeability to mannitol would be greater than that of lactulose in the normal BN rats, but the opposite was observed (Table VI-2). Thus, if mannitol did permeate via pores, this contribution must be small, since the apparent permeability was less than that of lactulose. However, the control BN rats were treated with CsA, which increased the intestinal permeability to lactulose and ^{51}Cr-EDTA compared to untreated BN rats (Sigalet DL: Cyclosporine effects on intestinal permeability. In Preparation). This may have been a non-specific stress response, or CsA may have had differential effects on the permeability of BN intestine to the markers used.

In the BN→Lew allogeneic SIT group there was increased permeability to each of the four markers used. This suggests that permeability via the tight junctions was increased. This likely has an immune basis, since it was not observed following syngeneic SIT. Similar increases in permeability have been noted with immune-mediated intestinal inflammation such as Crohn's disease (39). The observation of increased permeability to mannitol and PEG-400 but not ^{51}Cr-EDTA or lactulose in the syngeneic BN→BN and Lew→Lew groups suggests that these two probes have an additional pathway for uptake that is not available to ^{51}Cr-EDTA or lactulose. The tight junctions have been shown to be dynamic (40), and to respond to intraluminal solutes with a generalized increase in permeability, allowing the passive uptake of nutrients and markers such as mannitol (41). Thus, the increased permeability to mannitol and PEG-400 following SIT may represent a transplant-induced alteration in the tight junctions which is specific for these probes by nature of their size, location, or charge characteristics. The lack of increase in the in vitro passive uptake of L-glucose may represent a variation in permeability induced by the test conditions used. This study did not examine the status of the tight junctions between the intestinal epithelial cells directly; previous studies showed no changes in the short-term following SIT (8,42). More direct study is required, however, our findings demonstrate that if intestinal permeability is to be used as a marker for rejection (1,18) careful evaluation of baseline permeabilities must be done. The use of multiple markers may be useful.

The persistent malabsorption of dietary fat following SIT (Table VI-1) is not explained by the in vitro data (Table VI-4) in which the passive uptake of fatty acids was increased. However, this study did not examine the luminal phase of fat digestion, or fat transport by the lymphatics, both of which are important determinants of the absorption of dietary fat (43), and both of which may be affected by SIT (29,44). It has been shown that lymphatic regeneration is complete by 30 days posttransplantation (45), but this does not ensure that function is normal. Denervation may affect fat absorption by altering motility (44), and interrupting intestinal reflexes such as the ileal brake (46). Although fat malabsorption had minimal impact on the animal's status in the present study (probably due to the low-fat diet used: 4% of total calories), in the clinical situation with the typical high fat human diet it may be more problematic.

Denervation may also be the underlying cause of the changes in glucose transport and permeability observed. Acutely denervated bowel secretes water and chloride from the crypts due to a loss of sympathetic input (47-49); this persists after transplantation (6). While it is not clear what the secretory status of the intestine is following SIT, the animal must come to an equilibrium; the observed increase in sodium/glucose cotransporter activity may be compensatory.

Denervation could affect permeability indirectly by altering levels of intracellular cyclic-AMP, Ca^{++}, and protein kinase C which can affect junctional permeability (40,41,50,51). It has also been suggested that the sympathetic nervous system may affect the tight junctions directly (49). While these interactions require further study, they serve as a plausible explanation for the transport changes induced by SIT.

It is surprising that following the recovery from GVHD, allogeneic SIT had relatively little impact on nutrient handling by the intestine. The interaction between the immune system and intestinal function is poorly characterized, but immune events can certainly affect electrolyte and nutrient absorption in pathological states (52). The present study indicates that allogeneic SIT, with CsA immunosuppression, reduces dietary fat absorption, and increases intestinal permeability due to changes at the tight junctions. However, enterocyte function was not affect further.

The relatively normal in vivo absorption of nutrients following SIT suggests that adaptation of the bowel occurs following SIT. Despite a reduction in the glucose-stimulated Isc in all transplanted groups, and in the absorption of dietary fat, there was minimal impact on the absorption of dietary energy. The increase in mucosal transport capacity for glucose observed likely represents an adaptive response, which parallels the pattern of intestinal adaptation seen after intestinal resection, diabetes, or hyperphagia (33,53). While it is not clear from the present studies what the signals for this adaptation might be, it is significant the adaptation can occur following transplantation, and denervation. Feed intake, and humeral factors were not affect by SIT, which suggests that local signals controlled the adaptation seen.

SIT provides an interesting model for investigation of the links between the immune and nervous systems of the gut and how they affect nutrient absorption. The present study has demonstrated that despite alterations in function at the cellular level, the small intestine functions well after transplantation. The questions raised by this study will hopefully stimulate further work which will contribute both to our knowledge of normal gastrointestinal physiology and to the development of SIT as a useful clinical entity.

TABLE VI-1: Animal characteristics and nutrient absorption following allogeneic intestinal transplantation.

	CONTROLS	TRANSPLANTED ANIMALS		
	BN	BN→BN	Lew→Lew	BN→Lew
n	8	7	10	6
WEIGHT GAIN (Day 0-60)	38±3.7	10.6±3.2*	28±3.2	19.7±2.6*†
(% of basal) Day 28-60	9.2+0.9	5.1+1.4*	14.4+1.1	15.0+1.6
FEED INTAKE (mg feed·g body weight·day^{-1})	59±2	55±1	54±2.5	67±3
NUTRIENT ABSORPTION (% absorbed from diet)				
Energy Absorbed	83±0.3	81±0.7*	83±1	82±0.4
Fat Absorbed	78±0.9	76±0.9*	75±0.9*	74±1.2*†

mean±SEM

* p<0.05 versus BN controls

† p<0.05 versus Lew→Lew SIT

TABLE VI-2: Passive permeability of the small intestine following allogeneic intestinal transplantation.§

	CONTROLS	TRANSPLANTED ANIMALS		
	BN	BN→BN	Lew→Lew	BN→Lew
n	8	7	10	6
^{51}CrEDTA	5.6±0.7	5.1±1.0	4.8±0.6	12.7±1.1*†‡
Mannitol	1.9±0.3	4.8±0.2*	5.3±0.4*	5.9±1.3*
Lactulose	3.9±0.3	3.0±0.4	0.9±0.2	5.2±1.0*†‡
PEG400	8.2±1.7	37±8.7*	13.4±0.9	33±13*
Lac./Man.	2.5±0.4	0.62±0.24*	0.19±0.05*	1.02±0.19*†‡
^{51}CrEDTA/Man.	2.9±0.2	1.1±0.2*	0.9±0.1*	2.1±0.3†‡

§ urinary recovery % of orally administered marker

* p<0.05 versus BN controls

† p<0.05 versus (Lew→Lew) SIT

‡ p<0.05 versus (BN→BN) SIT

TABLE VI-3: In vitro nutrient uptake following allogeneic intestinal transplantation.§

	CONTROLS	TRANSPLANTED ANIMALS		
	BN	BN→BN	Lew→Lew	BN→Lew
n	8	7	10	6
JEJUNUM				
Vmax	30.6±4.1	73.3±10.4*	22.3±2.7	53.5±6.4*†
Km	2.6±1.3	11.5±3.0	3.5±1.5	8.7±2.5
ILEUM				
Vmax	16.2±2.2	39.5±7.6*	18.1±2.1	40.1±12.9
Km	2.6±1.5	7.9±3.3	6.9±1.0	20.8±11.7

§ Units: Vmax $nmol \cdot cm^2 \ mucosa^{-1} \cdot min^{-1}$

* $p<0.05$ versus BN controls

† $p<0.05$ versus Lew→Lew SIT

TABLE VI-4: In vitro fatty acid uptake following allogeneic intestinal transplantation.†

	CONTROLS	TRANSPLANTED ANIMALS		
LIPID	BN	BN→BN	Lew→Lew	BN→Lew
JEJUNUM				
12:0	20.2±3.0	15.2±1.8	6.5±1.2	10.0±1.6‡
18:0	1.1±0.2	Not done	0.5±0.1	1.3±0.6
18:1	0.9±0.2	1.7±0.3*	0.7±0.1	1.1±0.2‡
18:2	0.9±0.2	1.8±0.2*	0.6±0.1	1.3±0.3
18:3	0.8±0.2	1.3±0.1	0.5±0.1	1.1±0.2
Cholesterol	1.8±0.1	3.2±0.2*	0.9±0.1	1.6±0.2‡
ILEUM				
12:0	20.2±1.6	19.3±2.2	10.9±3.0	12.6±2.0
18:0	1.1±0.2	Not done	0.5±0.1	1.0±0.2
18:1	0.8±0.2	1.5±0.2	0.5±0.1	0.9±0.2
18:2	0.9±0.1	1.2±0.3	0.6±0.1	0.7±0.1
18:3	1.1±0.3	0.9±0.2	0.5±0.1	0.8±0.2
Cholesterol	2.8±0.6	5.2±1.2*	0.9±0.1	2.8±0.4‡

† Units are nmol/100 mg tissue/min

* $p<0.05$, transplant versus controls

‡ $p<0.05$, allogeneic transplant versus syngeneic transplant (BN→BN)

TABLE VI-5: Electrophysiological parameters following allogeneic intestinal transplantation.

	CONTROLS	TRANSPLANTED ANIMALS		
	BN	BN→BN	Lew→Lew	BN→Lew
n	8	7	10	6
JEJUNUM				
Glucose induced Isc (20 mM)	94.3±6.4	54.1±6.6*	29±5.0*	52.9±6.3*
Theophylline-induced Isc (5 mM)	27.2±4.9	25.0±4.9	25.0±3.8	26.4±3.9
Conductance (mS/cm^2)	30.2±1.2	22.8±1.4*	13.2±0.3*	18.5±0.7*
Resting Isc	37.8±2.2	39.2±4.7	24.9±2.8*	34.0±2.7
ILEUM				
Glucose-induced Isc (20 mM)	68.2±5.4	48.6±8.1*	44.0±1.3*	43.0±7.1*
Theophylline-induced Isc (5 mM)	44.1±3.4	48.1±2.2	33.3±2.1	45.1±3.6
Conductance (mS/cm^2)	17.9±0.9	23.4±3.0	15.1±0.4	20.9±2.7
Resting Isc	28.4±2.0	30.3±8.6	24.2±1.8	37.1±6.5

* $p<0.05$ versus BN controls

TABLE VI-6: Intestinal morphology following allogeneic intestinal transplantation.

TABLE VI-6A. JEJUNAL MORPHOLOGY

	No Tx	Syngeneic Tx		Allogeneic Tx
MORPHOLOGICAL PARAMETER	BN rats	BN→BN rats	Lew→Lew rats	BN→Lew rats
Crypt depth, μm	107±29	77±2	65±5	97±4
Villus height, μm	334±5	356±3	459±20	366±4†
Villus width at 1/2 height, μm	124±4	128±6	97±3	140±4†
Villus bottom width, μm	133±7	145±6	122±4	131±7
Villus thickness, μm	448±23	517±26	613±47	468±15†
Villus surface area μm^2/villus	434±6	516±12*	696±31	515±7*†
No. of villi/mm serosal length A	7.73±0.48	7.02±0.33	8.32±0.28	7.86±0.47
No. of villi/mm serosal length B	2.28±0.11	1.98±0.09	1.72±0.13	2.16±0.07†
No. of villi/mm^2 serosa	17.26±1.06	13.58±0.63*	13.58±0.46	16.82±1.02†‡
Mucosal surface area mm^2/mm^2 serosa	7.47±0.43	6.97±0.28	9.49±0.63	8.68±0.57

* $p<0.05$, transplant versus no transplant

† $p<0.05$, allogeneic transplant versus syngeneic transplant (BN)

‡ $p<0.05$, allogeneic transplant versus syngeneic transplant (Lew)

TABLE VI-6B. ILEAL MORPHOLOGY

MORPHOLOGICAL PARAMETER	No Tx	Syngeneic Tx		Allogeneic Tx
	BN rats	BN→BN rats	Lew→Lew rats	BN→Lew rats
Crypt depth, μm	81±3	83±4	71±5	99±4*†‡
Villus height, μm	217±11	326±15*	312±13	305±14*
Villus width at 1/2 height, μm	90±4	113±5*	86±2	125±5*†‡
Villus bottom width, μm	98±4	110±6	80±4	130±5*†‡
Villus thickness, μm	405±28	447±32	468±27	551±33*‡
Villus surface area μm^2/villus	249±13	417±18*	389±16	480±24*†‡
No. of villi/mm serosal length A	10.36±0.49	9.34±0.45	12.9±0.72	7.82±0.28*†‡
No. of villi/mm serosal length B	2.58±0.18	2.34±0.16	2.21±0.14	1.87±0.10*
No. of villi/mm^2 serosa	25.57±1.20	20.92±1.00*	27.57±1.55	14.19±0.51*†‡
Mucosal surface area mm^2/mm^2 serosa	6.40±0.53	8.67±0.47*	10.74±0.7	6.84±0.47†‡

* $p<0.05$, transplant versus no transplant

† $p<0.05$, allogeneic transplant versus syngeneic transplant (BN)

‡ $p<0.05$, allogeneic transplant versus syngeneic transplant (Lew)

FIGURE VI-1: Weight gain after allogeneic small intestinal transplantation.

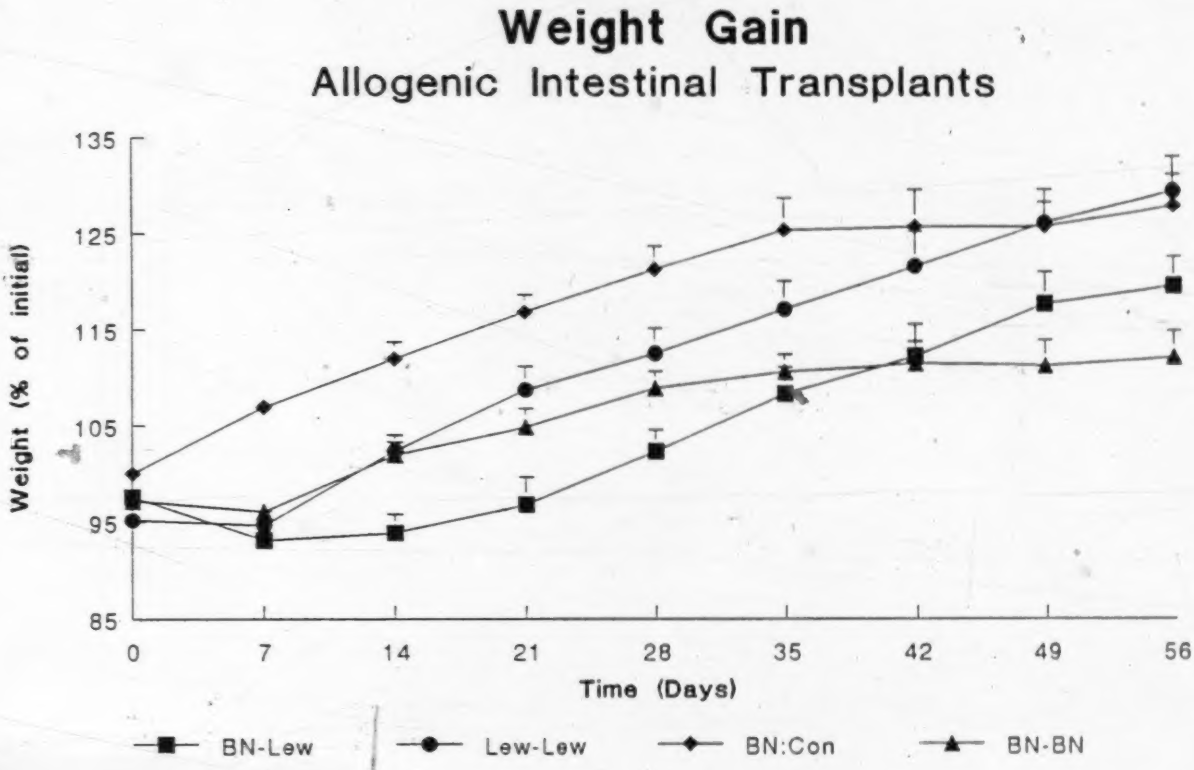
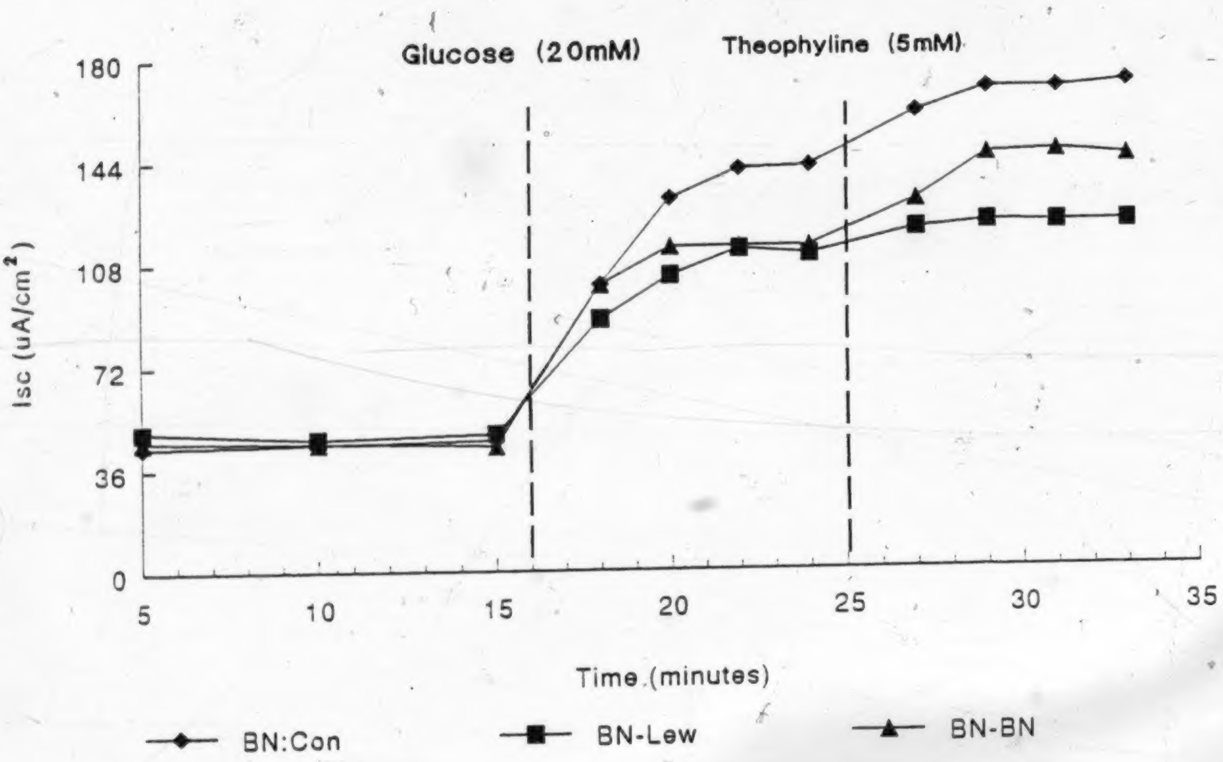


FIGURE VI-2: Short circuit current: changes with glucose and theophylline following allogeneic small intestinal transplantation.



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CHAPTER VII

THE EFFECTS OF CYCLOSPORINE AND INTESTINAL TRANSPLANTATION ON INTESTINAL FUNCTION IN THE RAT

INTRODUCTION

The preceding papers have examined various aspects of the impact of intestinal transplantation and cyclosporine A (CsA) treatment on small intestinal function. These findings will be reviewed and the mechanisms which may explain these observations discussed. The general effects of the experimental manipulations on the weight gain and nutrient absorption of the animals will be examined first, and then the sequential events associated with dietary nutrient digestion and absorption by the intestine will be discussed in turn. It is hoped that reviewing the findings in this way will facilitate formulation of investigations to answer the inevitable questions raised by these results.

CYCLOSPORINE'S EFFECTS ON NORMAL BOWEL

Chapter III reviews the findings regarding CsA's effects on intestinal function in normal rats, receiving 15 mg/kg subcutaneously on alternate days for 60 days. At this dose, there was no effect on the animals's body weight gain, or dietary nutrient absorption (Table III-1). However, as demonstrated in Chapter IV, high dose (30 mg/kg, subcutaneously on alternate days) parenteral CsA did reduce the body weight gain (Table IV-2). High dose oral CsA (30 mg/kg/day) caused a similar decline in weight gain, and both low (7.5 mg/kg/day) and high dose oral CsA treatment reduced the absorption of fat and total energy from the diet (Table IV-2).

Within the experimental conditions used, weight gain may reflect a decline in nutrient availability to the animal (primarily energy derived from starch) either from reduced feed intake, or reduced absorption of nutrients from the diet. Alternatively, a decline in the body weight gain may indicate a change in the basal metabolic rate, or metabolic "cost" of weight gain to the animal (1).

The effects of CsA given orally can be related to dietary nutrient absorption. Within the groups given CsA orally, there was no variation in feed intake, but high dose oral CsA treatment reduced total energy absorption from the diet by 7.8% (Table IV-2). This was due to a reduction in both fat and carbohydrate absorption. These studies did not examine the effects of CsA on nutrient digestion directly, however previous researchers have demonstrated reductions in both the volume of bile produced and the bile salt concentration with CsA administration (2-4). This in turn would reduce the efficiency of absorption of dietary fat (5), and could secondarily affect a number of other variables, such as the composition of the enterocyte membrane and the morphology of the intestine (6). The effect of CsA on the production and activity of intraluminal digestive enzymes is largely unknown; CsA has been shown to reduce the pancreatic exocrine output in the rat (7), and also to reduce intestinal brush border membrane disaccharidase activity in weanling rats (8). Thus there are a number of described effects of CsA which may reduce the efficiency of the intraluminal and brush border membrane phases of the digestion of nutrients.

NUTRIENT UPTAKE BY THE ENTEROCYTE

Nutrient transport by the enterocyte was examined in detail in the present studies. CsA, when given orally or subcutaneously, in high or low doses, adversely affected the maximal rate (Vmax) for active glucose uptake in all groups (except the high dose subcutaneous group, which had a large SEM, possibly suggesting experimental variability)

(Table IV-4). A reduction in Vmax indicates a reduction in the activity of the sodium-glucose cotransporter (9). The pattern of these changes was not straightforward; when CsA was given orally its effects on the Vmax for glucose did not appear to have a dose-response relationship (Table IV-4). When given by injection, low dose CsA reduced the Vmax for glucose uptake, but in high doses it increased the apparent Michaelis constant (Km*). It would be useful to know the local concentration of CsA in the tissue following these various dosage regimens. CsA has been shown to be metabolized by the intestinal mucosa (10), and has a variable concentration in tissues (11). CsA is metabolized by the liver and the metabolites excreted in the bile (12). Thus, the groups given CsA subcutaneously may be exposed to varying luminal concentrations of CsA, depending upon the rate of hepatic clearance of the drug. It may be that the hepatic toxicity of CsA alters its own metabolism, changing the resulting biliary concentrations of CsA metabolites, which then alters the activity of CsA's effects on intestinal function.

The mechanism(s) for CsA's effects on glucose uptake is not clear. In renal tubule cell culture, the sodium-glucose cotransporter activity declines immediately after exposure to CsA (13), suggesting a direct inhibitory effect. CsA also reduces protein synthesis in this same system after a 3-6 hour lag, suggesting down-regulation of transcription at the M-RNA level (14).

The alteration in the Km* for glucose noted in the group treated with high dose CsA given by injection is intriguing. The sodium-glucose cotransporter may be a heterogenous group of proteins, which can vary in expression depending on physiological controlling mechanisms (15). The alteration in Km* may represent a change in the population of the carriers induced by CsA. Alternatively, CsA has been shown to alter the phospholipid content on renal tubular cells (16), and in the present studies caused generalized changes in the intestinal uptake of fatty acids (Table IV-4), which implies an alteration in the composition of membrane of the enterocyte (5). If CsA does alter the composition of the enterocyte membrane, then this could secondarily alter the activity of all membrane bound proteins, including the sodium-glucose cotransporter (17).

CsA's effects on in vitro fatty acid uptake varied considerably with the route of administration (Table IV-4). Oral CsA had no significant effect on fatty acid uptake, which suggests that the reduction in absorption of dietary fat seen in these groups was due to other factors. These were most likely related to alterations in luminal emulsification and digestion caused by a CsA-induced reduction in bile salt production and bile volume, as discussed previously (2-4). The mechanism for the decreases in in vitro fatty acid and cholesterol uptake in the groups receiving CsA subcutaneously is not clear, but it suggests a change in the composition of the enterocyte membrane (18). These groups did not malabsorb dietary fat (Table IV-1), and so they would not have induced adaptive changes. The decreased uptake may reflect a direct effect of CsA on the enterocyte membrane, or possibly secondary effects such as the changes in prostaglandin synthesis as seen in the kidney (19).

The effects of CsA on morphology were dependent both on the route of administration of CsA and the dose of CsA given. There may have been an interaction between a primary effect of CsA on intestinal morphology and the adaptation process of the intestine. CsA had minimal effects on jejunal morphology, but had a greater impact in the ileum (Table IV-5), where adaptive responses are typically more pronounced (20,21). Animals receiving low dose oral CsA had an increase in villus size and mucosal surface area, while animals receiving high dose oral CsA demonstrated no change in morphology. The low dose oral group may have undergone an adaptive increase in surface area to compensate for the CsA induced reduction in glucose uptake, while higher doses of CsA may have prevented this response, resulting in a further impairment of dietary nutrient absorption (Table IV-2). The subcutaneously treated groups also had a dose-dependent response with low dose CsA reducing the villus size and mucosal surface area and high dose CsA given subcutaneously having relatively normal morphology. The factors which affected in vitro fatty acid uptake in these animals may also be affecting the morphology. Until the mechanisms underlying the effects of CsA on these nutritional parameters are understood, it may be difficult to discern how CsA is influencing intestinal morphology.

CsA also influences the nutritional status of the animals indirectly. The groups of animals receiving CsA by injection had no measurable decrease in energy or fat absorption from the diet (Table IV-2), and yet the group receiving high dose CsA (30 mg/kg q 2 days) gained less body weight than controls. There was a trend towards a decrease in feed intake with high CsA doses (Table IV-2), but this was not significant. It is unlikely that the 2 g/day (9.1 kCals/day or 11% of the total caloric intake) decline in feed intake noted between the low dose CsA (5 mg/kg q 2 days) and high dose CsA groups can account for the 37% decrease in weight gain noted for the latter group. This strongly suggests that high dose CsA given subcutaneously reduced the weight gain of these animals by increasing the metabolic "cost" of weight gain, or increasing the energy required for basal metabolic activities (the basal metabolic rate). The mechanisms for this are not clear from the present study, but it is known that CsA causes renal tubular atrophy, possibly by increasing the rate of intracellular protein degradation (22). This, coupled with the general reduction in protein synthesis seen with CsA, may represent a significant reduction in cellular metabolic efficiency with high dose CsA therapy.

Other interactions between CsA and intestinal function are possible. CsA's main toxicity in the kidney is a result of arteriolar vasoconstriction (19). CsA has recently been shown to cause similar reductions in the vascularity of the intestinal microcirculation (23). If CsA reduced the blood flow through the intestinal villus, this could affect all aspects of intestinal function (15). It also suggests possible therapy to prevent these effects; agents which inhibit production of certain prostaglandins have been useful in preventing CsA-induced nephrotoxicity (24,25), and could be effective in ameliorating the observed intestinal effects as well. CsA's main clinical utility lies in it's specific inhibition of the activity of the "helper" subset of T-lymphocytes (12). The interactions between the immune system and intestinal function are poorly understood at present, but there is no doubt that immunological events can profoundly affect intestinal function (26), and so the intestinal effects of CsA might be linked to its immunological activity.

The findings of this study suggest that CsA has a direct toxic effect on the activity of the sodium/glucose cotransporter of the enterocyte, which may be pharmacologic in nature. As well, CsA may decrease the number of cotransporter molecules. CsA appears to affect the membrane composition of the enterocyte when given subcutaneously, and likely has similar effects when given orally. Oral CsA reduces nutrient absorption from the diet, probably as a result of a combination of the changes in enterocyte transport activity discussed above, and alterations in luminal digestion caused by reduced bile output. These apparently widespread effects are not surprising, considering that CsA, acting through cyclophillin, could potentially affect any cell in the body by altering the signals between cell surface proteins and DNA expression by the nucleus (12,27,28). As the influence of the immune system on the gut becomes better understood and as we unravel the molecular events associated with CsA's activity, the sites of interaction between CsA and intestinal function should become more evident.

EFFECTS OF SMALL INTESTINAL TRANSPLANTATION ON INTESTINAL FUNCTION

The effects of small intestinal transplantation (SIT) on intestinal function were explored in chapters V and VI. The major findings were: 1) SIT has only a modest impact on dietary nutrient absorption and animal growth; 2) SIT does result in major changes in the transport properties of the intestine; 3) adaptation of transplanted intestine occurs; 4) the immune events associated with fully allogeneic transplantation (Brown Norway [BN] → Lewis [Lew]) and concomitant CsA treatment do not significantly affect the absorptive capacity of the bowel beyond the first 30 days following transplantation.

In examining the evidence for these conclusions, the weight gain and dietary nutrient absorption will again be discussed first. Following syngeneic SIT in Lew rats (Lew→Lew), untreated with CsA (CsA-), there was no change in the body weight gain, or the absorption of dietary total energy and fat compared with normal control animals (Table V-1). When syngeneic (Lew→Lew) SIT animals were treated with CsA (15 mg/kg subcutaneously, alternate days), there was a reduction in the absorption of dietary fat, but not total energy (Table V-1). Weight gain was not affected. This contrasts with the lack of effect of subcutaneously injected CsA on nutrient absorption by normal bowel (Tables III-1 and IV-2). When BN→BN sygeneic transplants (CsA+) were compared to normal BN animals (CsA+), there was a reduction in the absorption of dietary total energy and fat and body weight gain. Lew→Lew syngeneic SIT did not result in a drop in weight gain, and so this may reflect a differential effect in the impact of SIT on intestinal function, which is strain dependent.

The immune events associated with fully allogeneic transplantation do appear to have an additive detrimental effect on nutrient absorption following SIT. The weight gain of BN→Lew allogeneic SIT (CsA+) rats was reduced, compared to Lew→Lew syngeneically transplanted (CsA+) animals (Table VI-1, Figure VI-1). However, the rate of weight gain in the allogeneic SIT animals normalized 30 days following transplantation, and 14 days following the resolution of overt graft-versus-host-disease (GVHD). Dietary fat absorption was also further reduced in the allogeneic SIT group, when compared to sygeneic BN→BN SIT (Table VI-1, Figure VI-1).

How might these in vivo measures of nutritional status relate with the in vitro parameters of nutrient uptake noted in these studies? Within each of the two study groups, feed intake was relatively constant (Tables V-1 and VI-1). Energy absorption from this predominantly carbohydrate diet was also not affected. However, the in vitro Vmax for glucose was consistently increased following transplantation when compared to control animals of the same strain (Tables V-3 and VI-3). Because of the cotransport of sodium and glucose, an increase in the glucose-stimulated intestinal short circuit current (Isc) would have been predicted following SIT, but the converse was found (Tables V-5 and VI-5, Figures V-1 and VI-2). The normal resting Isc in these same groups suggests that a change in the activity of the sodium-potassium ATPase of the basolateral membrane cannot explain the reduction in the glucose-stimulated Isc. Although the net flux of sodium across the intestinal epithelium was not measured in these studies, an increase in "back" flux of sodium from the intercellular space to the intestinal lumen could explain the reduction in glucose-stimulated Isc noted in these studies. The increase in permeability to various probes which traverse the intercellular tight junctions (Tables V-2 and VI-2) argues in favour of this hypothesis. Alternatively, or in addition to these permeability changes, the conductance of the subepithelial portion of the bowel may also affect the intestinal short circuit current (39). Such changes have been noted following adaptation to small bowel resection, masking actual increases in enterocyte transport function (30).

The passive in vitro uptake of fatty acids and cholesterol was also affected by SIT (Tables V-4 and VI-4). This effect was most marked in the jejunum of BN→BN and the BN→Lew groups. The animals receiving transplants of BN intestine developed steatorrhea (Tables V-1 and VI-1); the increase in in vitro uptake of fatty acids may represent an adaptive response by the intestine. It is not clear from the present studies how SIT affects the absorption of dietary fat; certainly there are many potential sites for interaction (5).

Previous workers have implicated the disruption of the lymphatics as the cause of persistent steattorhea following SIT (31). Although the lymphatics have been shown to regenerate by 30 days following SIT, this does not assure that function is normal (32). Moreover, we saw a consistent pattern of reduced in vitro uptake of medium-chain fatty acids (dodecanoic [12:0]) following SIT, with increased uptake of long-chain (18:series) fatty acids (Tables V-4 and VI-4). This argues in favour of an adaptive increase in the uptake of the long-chain fatty acids, which depend on lymphatic transport, and a decrease in the uptake of the blood-borne medium-chain fatty acids. Previous workers have shown that denervation itself reduces fat absorption (33). This may be mediated by alterations in gastrointestinal motility (also seen with transplantation (34)), which could affect the emulsification of lipids. Alterations in the myoelectrical activity of the bowel could also affect the integration of gastric emptying, nutrient digestion and the secretion of intestinal enzymes (34). Other potential causes of decreased absorption of dietary fat include an increase in the "shedding" of intestinal mucosal cells, which increases the loss of the lipids of the cell membranes (5). Changes in in vitro fatty acid uptake have been linked with changes in the composition of the brush border membrane (5), and also to the activity of specific fatty acid binding proteins (35). These possibilities will require direct study to determine their relative importance following SIT.

The pattern of changes in permeability induced by transplantation is interesting. The permeation of the intestine by the probe molecules used is dependent upon the size (radius) of the probe (36), the number of openings in the intestinal mucosa (transmembrane pores and gaps in the tight junctions) (37), and on the physical solubility characteristics of the probe (38,39). Thus changes in permeability to a particular probe may reflect changes in the number of transepithelial passages available to it, or changes in the physical properties of the brush border membrane. Syngeneic Lew→Lew (CsA-) SIT resulted in a differential increase in permeability to the smaller probes: mannitol and ^{51}Cr-EDTA, with no change in lactulose, and a decrease in the permeability to PEG-400. The Lew→Lew (CsA+) group showed a similar pattern, but the permeability to ^{51}Cr-EDTA was increased further. BN bowel was also affected by CsA, and the BN controls (CsA+) had an increased permeability to ^{51}Cr-EDTA and lactulose when compared to normal BN animals (Sigalet DL, manuscript in preparation). This may represent an non-specific stress response induced by the CsA treatment, but more likely indicates a specific effect by CsA on the enterocyte tight junction in the control BN rats. The BN→BN (CsA+) group demonstrated an increase in permeability to mannitol which was similar to that seen following syngeneic transplantation of Lew bowel. Thus, CsA increased permeability to ^{51}Cr-EDTA in normal BN bowel, and in transplanted Lew bowel, implying an alteration in the tight junctions. Syngeneic SIT of BN or Lew bowel increased permeability to mannitol, but not lactulose. This suggests that SIT induces alterations in specific pores (possibly selective for mannitol) or tight junctions, but that these changes do not permit passage of lactulose. How then do we explain the lack of change in the in vitro uptake of L-glucose? It may be that the test conditions used for the in vitro studies of L-glucose altered the status of the tight junctions, which have been well characterized as dynamic structures (40). Alternatively, the permeability measurements may be influenced by alterations in the lipophilic properties of the enterocyte membrane (38). The lack of a change in the in vitro uptake of fatty acids in the syngeneic Lew→Lew SIT animals (Table V-2), despite the decline in PEG-400 permeability in this group would argue against this being a general explanation for the altered permeability to PEG-400 following SIT.

The changes in permeability following allogenic SIT (BN→Lew) (CsA+) are more straightforward (Table VI-2). The permeability of the bowel to the four probes used increased in concert, suggesting increased permeation by the pathway common to all four probes, the tight junction (38). The implication is that this represents ongoing immune-induced changes in the tight junctions of the small intestine, despite the normal morphology. Similar immune-mediated changes have been described in disease states, and have been related to breaks in the tight junctions induced by neutrophils (41).

The transport changes associated with SIT had surprisingly little impact on the overall well being of the animals. The in vitro uptake and morphological data strongly suggest that the intestine has adapted to the SIT-induced increase in permeability, and thus normalized the in vivo absorption of nutrients. The pattern of increased jejunal active glucose uptake seen in all groups following SIT is typical of the intestinal response to increased nutrient requirements, such as is seen after massive intestinal resection, diabetes, irradiation, and hyperphagia (20,21,42). The one group which did not increase the active transport of glucose was the (Lew→Lew) (CsA+) group (Table V-3), presumably as a result of the CsA treatment that group received. However, this group absorbed energy from the diet normally (Table V-1). The increase in ileal villus size and overall mucosal surface area noted in this same group when compared to controls and Lew→Lew (CsA-) SIT animals (Table V-6), is a typical morphological adaptive response. The increased surface area available for nutrient absorption, improves the extraction of energy from the diet, overcoming a reduced transport capacity per unit mucosal surface area (21). The signals for the adaptive response generally have been thought to be neural, hormonal, or luminal in nature (20,21). The observed adaptation of denervated bowel, in animals receiving identical quantities of food, suggests that the signal in this case was local in nature.

Having reviewed the changes in transport function associated with SIT, it is useful to consider what the initial stimulus for these alterations might be. Denervation, perioperative hypoxia, immune responses, and changes in enteric hormone levels are potential causes. Perioperative hypoxia damage is unlikely to be a factor, because the functional studies were performed long after the known repair period for damage of this level (43). Immune responses cannot initiate stimulus for changes in transport capacity, since the same pattern of changes in permeability and transport function were observed in syngeneic and allogeneic intestinal transplants. Moreover, the fully allogenic transplants (BN→Lew, CsA+) had no greater change in the in vitro transport properties than the BN→BN (CsA+) group (Tables VI-3 and VI-5). The enteric hormones that have been examined following SIT (cholestokinin, neurotensin, vasoactive intestinal peptide, somatostatin, and substance P) had a near normal distribution and normal levels (44,45), and so are unlikely to be triggering changes in intestinal function.

Denervation has been shown to induce alterations in fat absorption which mimic the changes noted following SIT (33). The acutely denervated gut secretes chloride, and this condition persists after SIT (46,47). As noted previously, the intestinal epithelial tight junctions respond to a variety of stimuli, which include luminal solutes, the rate of Na-coupled nutrient transport, and intracellular signals such as cyclic-AMP, calcium, and protein kinase C (40). It has been further suggested that the sympathetic nervous system may affect the permeability of the tight junctions directly (48). Thus, the denervation induced by SIT may result in an increase in permeability of the intestine, leading to increased back-diffusion of nutrients from the paracellular space into the intestinal lumen. The enterocyte adapts to this by increasing the transport capacity of the brush border membrane, thus preserving overall nutrient absorptive function. Alternatively, denervation may induce changes in the secretion of chloride from the crypts, changing net electrolyte flux, and secondarily affecting the intestinal permeability. It is interesting to note that the moderate increases in permeability noted do not have a major effect on nutrient absorption, which is as would be predicted from the model of normal solute drag of nutrients (40).

There are a number of potential areas of further study which are suggested by these findings. The central issue is the determination of the mechanisms for the observed effects of CsA and SIT on the transport characteristics of the bowel. Our findings suggest that CsA may be affecting glucose uptake directly, and fat absorption through alterations in the brush border membrane and the intraluminal phases of digestion. To examine these questions, it would be useful to examine the effect of acute exposure CsA on the function of the intestinal mucosa. This could be done by modifying our currently used methods: in vitro active glucose uptake could be assessed with and without a pharmacologic concentration of CsA in the incubation fluid of intact tissue (42), or using vessicle preparations (36). The acute effects of CsA on intestinal Isc could be determined by the addition of a similar pharmacologic concentration to the Ussing chamber apparatus, and measuring the effect on Isc directly (10). The chronic effect of CsA on the transport properties of the enterocyte could be further explored by direct determinations of the lipid composition of the brush border membrane, the activity of the sodium-potassium ATPase of the BLM, and the number and distribution of sodium-glucose cotransporters (36,42,49). The effect of orally administered CsA on bile flow and bile salt production could be measured directly (2-4). It is interesting to note that the previous reports regarding CsA's effect on hepatic function used parenterally administered CsA (2-4). Given the recent description of the metabolism of CsA by the intestinal mucosa (10), these studies may not apply to orally administered CsA. Finally, the impact of CsA on the net flux of nutrients could be determined directly by measuring transmural fluxes (50).

The previously described influence of the prostaglandins on CsA's toxicity has led to the therapeutic use of prostaglandin analogues, and dietary fish oil, which influence prostaglanding metabolism (24,25). The influence of these therapies on CsA-induced intestinal malabsorption could be examined. This may then prove to be useful clinically, as CsA therapy for inflammatory bowel disease becomes more widespread (51).

Given the rapid proliferation in new drugs for immune suppression (28), there are a number of alternatives to CsA which may soon be generally available. Since these new drugs may share common mechanisms of action with CsA, they exhibit similar toxicity to the intestine, and so should be evaluated prior to use with SIT.

A similar approach would be required to determinine the mechanisms underlying the effects of SIT on bowel function. Of particular importance would be the determination of net transmural sodium and glucose fluxes following SIT. The effects of SIT on the tight junction permeability are a central issue in the explaination of the reduction in glucose-stimulated Isc noted after SIT. These are amenable to direct morphologic investigation (38), as well as in vivo assesment using a variety of probes Na clip is taken in vesicles. Similarly, we have suggested a possible influence of denervation on the permeability of the small intestine. This could be investigated directly as well by denervating bowel in situ and then determining the transport and permeability changes induced (33). The specific effects of SIT on the sodium-glucose transporter should be examined, to determine the nature of the increase in transport capacity for glucose induced following SIT (49,50).

Denervation of the intestine should induce alterations in sensitivity to neurotransmitters. This can be tested by examining the Isc response to the various transmitters and their analogues. If altered neural sensitivity is a cause of transport abnormalities following SIT, then pharmocologic blockade may be a useful therapy. This too can be examined using Ussing chambers.

The question of the reduced absorption of fats following SIT and CsA may require investigation in vivo (5). For example, using radiolabelled tracers in vivo, the efficiency of complex macronutrient absorption can be studied. The time course of the intravenous appearence of the marker correlates with the efficiency of the various steps of nutrient digestion and absorption (5). Alternatively, in vivo loops could be used to examine the carrying capacity of the transplanted lypmphatics. Finally, the intracellular processing of absorbed fats can be examined in vitro, and the effects of SIT and denervation on fatty acid handling, and excretion as lipoproteins in the lymph determined.

The studies reviewed herein have suggested that CsA treatment and SIT induce a spectrum of previously changes in intestinal function that were previously not appreciated. It is hoped that the results of these studies will be useful in planning future investigations into these areas, with the ultimate goal of improving our ability to manage patients with gastrointestinal diseases of all kinds.

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